

BIOLOGICAL TREATMENT OF 2,4,6-TRINITROTOLUENE (TNT),
HEXAHYDRO-1,3,5-TRINITRO-1,3,5-TRIAZINE (RDX), AND
OCTAHYDRO-1,3,5,7-TETRANITRO-1,3,5,7-TETRAZOCINE
(HMX), AND THE HYDROLYSATES OF RDX AND HMX

BY

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To Lynda, my eternal love,
and to Mike Reed, you will be missed.

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Abstract of Dissertation Presented to the Graduate School
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Biological treatment of 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) was investigated in anaerobic systems for comparison with RDX and HMX treatment in activated sludge (AS) systems following hydrolysis. The AS systems (0.74 % total solids) denitrified 17 and 19 additions of RDX and HMX hydrolysate waste, respectively, over 24 days. The AS reactors required 8 hours (RDX hydrolysate) and 5 hours (HMX hydrolysate) to remove 95.5% and 93.9% of hydrolysate nitrite (500 ppm).

Anaerobic treatment of TNT, RDX, and HMX was investigated using biochemical methane potential (BMP) and high anaerobic solids assays (HAS) with and without TNT acclimation.

Anaerobic sludge was generated from reactors charged with waste activated sludge and horse manure (3:2 ratio based on dried solids). Reactors were maintained at 35 C, had a hydraulic residence time of 24 weeks and were fed Purina Dog Chow at a rate of 0.85 g/L/day.

Total gas generation data from BMP assays indicated no sludge acclimation effect within the TNT, HMX and control treatments. A decrease in gas production rates was noted for RDX treatments of TNT acclimated sludge solids ($P = 0.026$).

Lower total gas yields were observed in TNT treatments when compared to control treatments ($P < 0.001$). Raw RDX and HMX hydrolysate treatments resulted in lower gas evolution rates compared to control treatments ($P < 0.0001$). Activated sludge pretreatment of RDX and HMX hydrolysates eliminated these differences.

The TNT acclimation of sludge solids increased TNT transformation rates in HAS assays ($P < 0.001$). Acclimated systems required 30 minutes to transform 59 ppm TNT versus 43 ppm in non-acclimated systems. Additionally, acclimated systems removed aminodinitrotoluene byproducts in 120 minutes versus 960 minutes for the non-acclimated systems.

No sludge effect was noted for RDX and HMX treatments. The RDX treated reactors (100 ppm dose) transformed 81.8 ppm RDX in 960 minutes. The HMX treated reactors (100 ppm dose) transformed 69.4 ppm HMX in 5760 minutes. Comparison of AS to HAS systems indicated a greater treatment

potential for RDX (18 times) and HMX hydrolysates (140 times) in the AS systems with hydrolysis.

CHAPTER 1 INTRODUCTION

Background

The last century of the second millennium saw significant manufacturing of high-energy explosive compounds to support military forces around the world. Unfortunately, it has only been in the last 20 to 30 years that we have begun to pay attention to the environmental problems resulting from the uncontrolled discharge of various primary and secondary explosive compounds.

Over one million cubic yards of contaminated soil requiring treatment are believed to exist on US army bases throughout the world (Lotrario et al., 1997). Existing sites with contamination levels ranging from a few parts per million that require little or no treatment to sites that contain explosives up to 50 percent by mass that require very careful handling have been identified (Binks et al., 1995).

At the end of 1992, the United States Department of Defense had a total accumulated demilitarization inventory of 313,000,000 kg of high explosives and was continuing to demilitarize munitions at a rate of 56,000,000 kg per year (USAEC, 1988). Additionally, in 1992, the U.S. Department of Energy estimated that it would generate 50,000 kg of high explosives per year requiring disposal through the year 2000 (USAEC, 1988).

These decommissioned high energy compounds must be disposed of in a manner that limits environmental releases. Additionally, contaminated sites

must be remediated before migration of the contaminants occurs.

Treatment Approaches

Open-pit burning has traditionally been used for disposal of decommissioned munitions in quantities of up to 27,000 kg per disposal event. Thermal destruction of munitions in this manner results in the release of significant quantities of the parent compounds and decomposition byproducts. In 1992, the US Department of Defense disposed of almost 50,000 kg of decommissioned munitions through open-pit burning.

Incineration uses a more controlled method of thermal destruction that controls burn temperature, fuel feed rate and oxygen mixture to optimize the destruction of organic compounds and minimize environmental releases. Large scale implementation of incineration technology has been hindered by negative public perception.

Biological treatment of high-energy compounds has been investigated by numerous researchers. Studies range from investigations of particular microorganisms under controlled laboratory conditions to enhancing native biological populations in situ.

The nitroaromatic 2,4,6-trinitrotoluene (TNT), and nitramines hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) show little susceptibility to degradation and mineralization under aerobic conditions without prior chemical pretreatment. The formation of highly toxic and recalcitrant nitroaromatic azoxy compounds during aerobic treatment of TNT and various other nitroaromatics significantly reduces its

usefulness as an effective treatment approach (Spain, 1995; Carpenter et al., 1978).

Anaerobic microorganisms show significant promise as an effective treatment approach for the destruction of many nitrated explosive compounds. Under appropriate pH, temperature, nutrient and oxidation/reduction conditions, TNT, RDX and HMX degradation by various species of anaerobic microorganisms has been observed. (Boopathy, et al., 1992; McCormick, et al., 1976; Ederer, et al., 1997; Spain, 1995).

Previous exposure to explosive compounds may enhance degradation rates through acclimation of the microorganisms to the particular waste stream. Acclimation of microbial populations to a specific waste stream has been observed within the hazardous waste industry for years (Boyd and Sheldon, 1984).

An alternative to direct biological treatment of RDX and HMX uses high temperature alkaline hydrolysis to achieve cleavage of the ring structure. The benefits of this approach are that large quantities of explosives can be rapidly degraded with elimination of the explosion threat. Hydrolysis generates a waste stream containing high nitrite levels and a variety of organic compounds including acetate, formate, and formaldehyde (Saupe et al., 1998; Heilmann et al., 1996).

Exposure of TNT to sodium hydroxide results in the formation of azoxy compounds and does not result in the opening of the aromatic ring (Heilmann et al, 1996). This potentially limits the use of this technology on munitions mixtures containing TNT or at sites where TNT or other nitrated aromatics are present.

The treatment of the waste stream generated by RDX and HMX hydrolysis may be accomplished efficiently and inexpensively through biological means. The use of activated sludge technology for the treatment of nitrate and nitrite containing solutions has been extensively investigated within the wastewater industry. Application of standard design procedures for activated sludge systems would permit investigation of this technology for the treatment of RDX and HMX hydrolysates as an alternative to direct treatment of the parent compounds.

Purpose of Study

Investigations of US military installations in the United States and around the world indicate that load, pack, and assembly operations have discharged significant amounts of high explosives have been discharged to the environment. Alternatives to current disposal technologies are required to limit the uncontrolled release of these compounds.

Research was undertaken to investigate the acclimation of anaerobic sludge solids to TNT in order to enhance TNT, RDX, and HMX transformation rates. System effects and transformation rates for investigational compounds were examined for TNT acclimated (experimental) and non-acclimated (control) sludge solids using a modified biochemical methane potential (BMP) assay and a high anaerobic solids (HAS) assay. Procedures for the operation of anaerobic reactors were developed for future investigations and applications of the technology. Activated sludge treatment of RDX and HMX was investigated after alkaline hydrolysis for evaluation as an alternative treatment method.

Rationale

Numerous researchers have investigated the degradation of TNT, RDX and HMX under various environmental conditions by a variety of microorganisms. The most successful approach has been the use of anaerobic and facultative anaerobic microorganisms under controlled laboratory conditions (Boopathy and Kulpa, 1992; Boopathy and Kulpa, 1993; Boopathy and Manning, 1996; Boopathy, Manning, and Kulpa, 1997; Boopathy, Wilson, and Kulpa, 1993; Costa, Boopathy, and Manning, 1996; Fiorella and Spain, 1997; Fuller and Manning, 1997; Funk et al., 1995; Kahn, Bhadra, and Hughes, 1997; Lewis et al., 1996; Marvin-Sikkema and de Bont, 1994; McCormick et al., 1976; Montpas et al., 1997; Spain, 1995). Some of the microorganisms studied partially reduced TNT while others completed the reduction of TNT to triaminotoluene (TAT) under specific conditions.

Many of these researchers recommended a mixed-culture anaerobic approach in order achieve complete reduction of TNT to toluene followed by mineralization. Anaerobic reactors (1.5 L) were used with an activated sludge and horse manure microbial seed to permit the development of a biologically diverse mixed-culture system.

Researchers in the chemical industry have observed the adaptation of anaerobic microbial populations to a specific waste stream for decades . Investigators have successfully degraded many highly toxic aerobically recalcitrant compounds through repeated additions to captive microbial population under the appropriate operating conditions (Boyd and Sheldon, 1984).

Sludge acclimation to TNT was investigated as a means of further enhancing transformation rates of high explosives.

High-solids anaerobic digestion was chosen to maximize transformation rates. Previous investigations at the University of Florida indicated a direct relationship between anaerobic solids content and the rate of TNT transformation (James, 1999).

Objectives

The objectives of this research were as follows:

1. Develop a startup procedure for high-solids anaerobic reactors using an activated-sludge and horse-manure microbial seed.
2. Develop a procedure for acclimating anaerobic sludge solids to TNT.
3. Apply the activated sludge treatment process to hydrolysate treatment for nitrite and COD reduction.
4. Develop a standard high anaerobic solids (HAS) assay to quantify transformation rates for investigational compounds.
5. Investigate the effects of a) TNT, b) RDX, c) HMX, d) raw and denitrified RDX hydrolysate and d) raw and denitrified HMX hydrolysate on ultimate gas yield and gas evolution rates using a modified BMP assay.
6. Determine transformation rates for a) TNT, b) RDX c) HMX and d) an equal mass mixture using a high anaerobic solids assay procedure with TNT acclimated and non-acclimated sludge solids.
7. Statistically evaluate the effect of TNT acclimated and non-acclimated sludge solids on BMP ultimate gas yield and gas generation rates, and

HAS explosive transformation rates.

8. Assess the treatment potential of anaerobic sludge solids and activated sludge solids for comparative analyses.

CHAPTER 2 LITERATURE REVIEW

Energetic Materials

Classifications

Explosive compounds decompose rapidly after mechanical or thermal ignition with the evolution of significant quantities of gas and thermal energy. They are categorized as high explosives and low explosives depending on their energetic properties.

Low explosives undergo relatively slow combustion after initiation while evolving large volumes of gas in a known and controllable manner. The low explosive classification includes compounds such as black powder (potassium nitrate, sulphur, coal) and nitrocellulose.

High explosives react violently after reaction initiation, rapidly evolving large quantities of heat and gas. They comprise a majority of explosive contaminated sites and a significant fraction of decommissioned munitions awaiting disposal (USAEC, 1988). High explosives are divided into primary and secondary compounds based on their susceptibility to initiation.

Primary explosives are the most sensitive to initiation by heat or shock. Compounds such as mercury fulminate and lead azide are used in small quantities to initiate reactions in secondary explosives.

Secondary explosives are mainly composed of the nitrates, nitroaromatics, and nitramines. They are more stable than primary explosives in terms of sensitivity to thermal or mechanical initiation. Their stability in conjunction with their high power has resulted in a wide variety of military applications.

Three of the most commonly used secondary explosives are 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetraazocine (HMX). They are found frequently in military munitions in various mixture ratios depending upon the application.

Chemistry and Production of TNT

The nitroaromatic compound TNT consists of a benzene ring with a methyl group at the 1 position and nitro groups at the 2, 4 and 6 positions. The chemical formula of TNT is $C_7H_5N_3O_6$ and its chemical structure is presented in Figure 2-1. TNT is also referred to in the literature as 1-methyl-2,4,6-trinitrobenzene, 2-methyl-1,3,5-trinitrobenzene, alpha-trinitrotoluene, alpha-trinitrotoluol, tolit, trilit, and trotyl oil (USAEC, 1988).

The molecular weight of TNT is 227.13 g/gmole and it has a specific gravity of 1.654. It has a melting point of 80.1°C and a boiling point of 240°C at which point it explodes. At 20°C TNT is a solid composed of odorless monoclinic white needles with a solubility in water of approximately 130 ppm (Ryon et al., 1984). It is extremely soluble in a variety of organic solvents including acetone, benzene, alcohol and ether.

The production of TNT occurs through the nitration of toluene using a

mixture of sulfuric acid and nitric acid in a stepwise fashion. By increasing the reaction temperature and acid concentrations, nitro- groups are bonded to toluene successively forming mononitrotoluene (MNT), dinitrotoluene (DNT), and trinitrotoluene (TNT). Impurities formed during the production process include various TNT isomers such as 2,3,4-trinitrotoluene and 2,3,5-trinitrotoluene, partially nitrated compounds like mononitrotoluene and dinitrotoluene, and various oxidation products like nitrobenzoic acid and nitrocresol. Refinement of the final product results in the removal and disposal of many of these impurities (Nay et al, 1974).

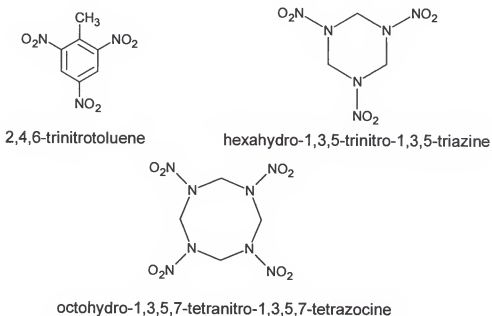


Figure 2-1. Chemical Structures of TNT, RDX, and HMX

Wastewater from TNT production is classified as yellow or red water depending upon its source. Yellow water is the waste acid solution from TNT production and may contain significant quantities of TNT and byproducts (Nay et

al., 1974). Red water contains sulfonated TNT isomers.

Wastewater generated by load, assembly and pack (LAP) facilities is referred to as pink water. Pink water contains a variety of compounds including 1,3,5-trinitrobenzene, 2,4,6-trinitrobenzaldehyde ADNT, DANT, TNT and numerous nitroaromatic azoxy compounds (Richter-Torres, 1995). Improper disposal of these wastewaters has resulted in significant contamination at many manufacturing and processing facilities (Binks et al., 1995).

Chemistry and Production of RDX and HMX

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is a nitramine consisting of a 6-atom carbon/nitrogen triazine ring with nitro- groups bonded to the ring nitrogen atoms. RDX has a chemical formula of $C_3H_6N_6O_6$ and its chemical structure is presented in Figure 2-1. Synonyms for RDX include cyclonite, 1,3,5-triaza-1,3,5-trinitrocyclohexane, 1,3,5-trinitrohexahydro-1,3,5-triazine, cyclotrimethylene-nitramine, hexogen and hexolite.

The high explosive compound RDX has a molecular weight of 222.13 g/gmole and a melting point of 204°C. However, it decomposes before melting (Ryon, et al, 1984). At 20°C RDX is a white solid with a density of 1.82 g/mL and a solubility in water of 38.4-38.9 ppm (Ryon et al., 1984). It is slightly soluble in methanol, ether, ethyl acetate and glacial acetic acid.

Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetraazocine (HMX) is also a member of the nitramine class of explosive compounds. Structurally, HMX consists of an 8-atom carbon/nitrogen tetraazocine ring with nitro- groups bonded to the ring nitrogen atoms. The chemical formula for HMX is $C_4H_8N_8O_8$ and its chemical

structure is presented in Figure 2-1.

The molecular weight of HMX is 296.17 g/gmole. It melts at 286°C, decomposes before boiling and has a solubility in water of 0.14 ppm at 83°C (Ryon et al., 1984).

The nitramine compounds RDX and HMX are both produced using similar processes and are always found as contaminants in the final product when either is produced. The most common method used for RDX production is the Bachmann Process which involves reacting hexamine with nitric acid, ammonium nitrate, glacial acetic acid and acetic anhydride. The Bachmann process yields a product with only an 8 to 12% HMX impurity level. The high explosive compound HMX is produced using the same process at a higher reaction temperature. The HMX yields range from 55 to 60% with the remainder consisting of RDX (USAEC, 1988).

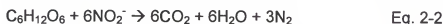
Biological Processes

Aerobic and Anoxic Processes

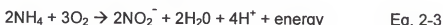
Aerobic reaction kinetics make it the most effective and efficient approach for many organic waste streams. The use of oxygen as an electron acceptor permits many microorganisms to maximize the energy yield from the oxidation of a variety of organic compounds.

Facultative anaerobic microorganisms preferentially use oxygen as an electron acceptor but can also use nitrate or nitrite when sufficient oxygen is not available. The use of nitrate and nitrite as electron acceptors results in the

generation of molecular nitrogen (N_2) which is easily removed from the system. Equations for nitrate and nitrite reduction to N_2 are presented in Eq. 2-1 and Eq. 2-2 below.



Chemoautotrophs may oxidize ammonium to nitrite under aerobic conditions as presented in Eq. 2-3. Nitrite may be further oxidized to nitrate as shown in Eq. 2-4. The two oxidative steps are carried out by *nitrosomonas* and *nitrobacter* respectively to obtain energy.



Activated sludge systems take advantage of these nitrogen conversions by cycling the system through aerobic and anoxic stages. This eliminates ammonium, nitrate, and nitrite nitrogen in an extremely efficient manner. Additionally, reduction of the wastewater BOD occurs as organic compounds are oxidized.

Calculation of BOD requirements for nitrate and nitrite reduction permits comparison with the wastewater's available organic compounds to ensure that sufficient BOD is present to maintain healthy microbial population. Systems with insufficient BOD must receive a supplemental carbon source or complete reduction of nitrate and nitrite will not occur.

Some organic compounds are not amenable to aerobic oxidation. Many of these aerobically recalcitrant compounds can be degraded when the oxidation

reduction (redox) potential of the system is sufficiently reduced.

Oxygen presence results in oxidation/reduction (redox) potentials greater than 400 millivolts (mV). Anoxic systems include redox potentials in the nitrate reduction range from 250 mv to the upper end of the aerobic range. The redox potential of anaerobic systems ranges from the bottom of the anoxic range to -350 mv (Mitsch and Gosselink, 1993).

Anaerobic Stabilization

Anaerobic microorganisms thrive in temperatures ranging from mesophilic (5°C to 50°C) to the thermophilic (55°C to 70°C). Hobson et al. (1981) and Pfeffer (1974) indicate that the optimum temperature values for anaerobic digestion are 40°C to 44°C in the mesophilic range and 55°C to 60°C in the thermophilic range. Experiments are rarely performed in the thermophilic range because of operational problems resulting from bacterial sensitivity to temperature fluctuations at higher temperatures (Hobson, 1981). The optimum pH for anaerobic digestion is around 7.0. The pH must be maintained above 6.0 or inhibition of methanogenic bacteria will occur (Hobson, 1981).

Anaerobic stabilization of organic compounds involves a complex series of biochemical reactions performed by numerous species of microorganisms. Earle et al. (1990) classifies the stages of organic waste transformation as hydrolysis, acidogenesis, link processes, and methanogenesis.

Hydrolysis of complex organic compounds such as proteins, fats, and carbohydrates by hydrolytic bacteria results in the formation of simpler compounds (Smith et al., 1988). Hydrolysis is followed by acidogenesis during

which these simpler compounds are fermented to organic acids and alcohols followed by conversion to various volatile fatty acids. Link processes result in the formation of acetate, hydrogen, and carbon dioxide from Stage 2 products.

During methanogenesis, link process products are oxidized by carbon dioxide reducing bacteria. This results in the formation of carbon dioxide, methane and various trace gases including hydrogen and hydrogen sulfide. Methane concentrations generally range from 50% to 60% with carbon dioxide constituting the remaining 40% to 50%. Trace gases usually constitute less than 1% of total gas production (Sweeten and Reddell, 1985).

Transformation of TNT, RDX, and HMX

Nitro- Group Reduction

Biological transformation of nitroaromatics and nitramines begins with the nitro group substituents. The chemical structure of this group leaves it highly susceptible to reductive attack. This is especially true of aromatic nitro groups.

Oxygen and nitrogen compete for electrons in the nitro- group. The higher electronegativity of the oxygen atoms results in a polarization of the nitrogen-oxygen bond. The nitrogen atoms' positive charge and the nitro- groups' high electronegativity results in readily reducible nitro- groups (Spain, 1995).

The methyl group bonded to the aromatic ring in TNT further decreases the electron density of the ring. Additionally, it increases the electron withdrawing force on the nitro- group para to it and increases the probability of para nitro group reduction (Preuss and Rieger, 1995). This results in the

fractional formation of 2-amino-2,6-dinitrotoluene to 4-amino-4,6-dinitrotoluene less than expected (McCormick, 1976).

Biological reduction of each nitro group in the TNT molecule proceeds through the nitroso- and hydroxylamino- intermediates to the amino form as shown in Figure 2-2. Strictly anaerobic conditions result in the eventual reduction of all three TNT nitro- groups to amino- groups when the appropriate microorganisms and environmental conditions are present (Spain, 1995).

In aerobic systems, the hydroxylamino- intermediates may be non-enzymatically oxidized forming various insoluble nitroaromatic azoxy compounds (McCormick, 1976). Azoxy compound formation appears as an insoluble dark reddish brown precipitate. The formation of azoxy compounds is undesirable and indicates unfavorable reaction conditions.

Formation of azoxy compounds also occurs in anaerobic systems when the pH is greater than 7.0 (Spain, 1995). Azoxy compounds which have been found to form under these conditions include 4,4'-dinitro-2,6'-azoxytoluene, 4,2'- dinitro-2,4'-azoxytoluene, and 2,2'-dinitro-4,4'-azoxytoluene (Walker and Kaplan, 1992).

Under the appropriate conditions in anaerobic systems, the reduction of the nitro groups continues through the nitroso- and hydroxylamino- intermediates to the amino form. The amino form has a lower electronegativity than the indicated nitro form, resulting in an increase in the electron density of the aromatic ring. The increased ring electron density decreases the ease at which the remaining nitro group substituents can be removed, resulting in reduced

transformation rates (Spain, 1995). Complete reduction of all TNT nitro groups in TNT to amino groups results in the formation of triaminotoluene which has a much higher electron density within the aromatic ring than the starting nitroaromatic compound.

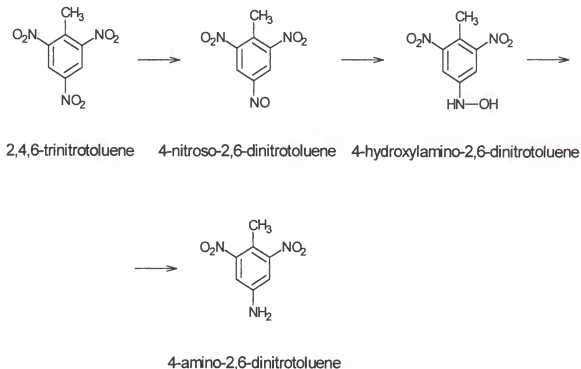


Figure 2-2. TNT reduction to ADNT showing nitroso- and hydroxylamino-intermediates.

Biological reduction of RDX and HMX nitro- groups proceeds through the nitroso- and hydroxylamino- intermediates to the amino forms as seen during TNT reduction; however, reduction of RDX and HMX to this point appears to destabilize the molecule resulting in the opening of the ring structure and the formation of simpler organic compounds (Spain, 1995).

Transformation Pathways

Boopathy and Kulpa (1992), Boopathy et al. (1996), Crawford (1995), Gorontzy et al. (1996), and Preuss and Rieger (1995), have proposed TNT transformation pathways by anaerobic microorganisms. The TNT molecules' nitro groups are sequentially reduced forming aminodinitrotoluene's followed by diaminonitrotoluene's and a final nitro group conversion forming triaminonitrotoluene as shown in Figure 2-3. Beyond this point consensus differs as to the degradation pathway.

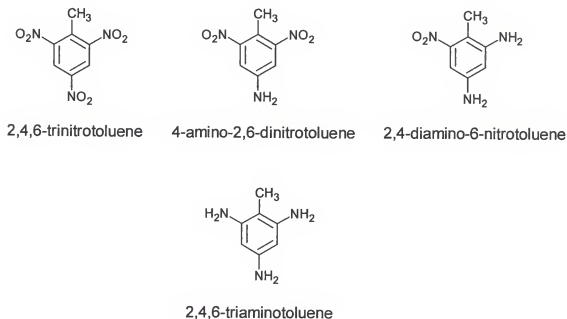


Figure 2-3. Reduction of 2,4,6-trinitrotoluene (TNT) to triaminotoluene (TAT).

Boopathy et al. (1996), and Preuss and Rieger (1995) proposed the transformation of triaminotoluene to toluene by reductive deamination. Toluene can be degraded biologically and transformation of TNT to this point should ultimately result in mineralization (Boopathy, 1996).

Crawford (1995) suggested that a second transformation pathway exists as shown in Figure 2-4. Hydrolytic displacement of the amino groups may occur forming polyphenols. This is a non-enzymatic conversion of triaminotoluene to trihydroxytoluene and is reversible; however, according to Crawford, equilibrium favors almost complete conversion to the deaminated compounds. Simpler phenols like p-cresol are then formed through reductive dehydroxylation of the polyphenols followed by fermentation to volatile organic acids.

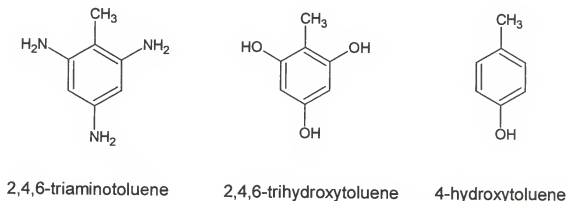


Figure 2-4. Hydrolytic displacement of triaminotoluene (TAT) amino groups forming polyphenols followed by reductive hydroxylation.

Microbiological Investigations

Numerous aerobic, facultative anaerobic and anaerobic microorganisms may transform TNT, RDX, and HMX. Investigations under a variety of laboratory conditions have included the microorganisms *Clostridium* spp., *Veillonella alcalescens*, *Desulfovibrio* spp., *Methanococcus* sp, *Escherichia coli*, *Salmonella typhimurium* *Serratia macresens*, and *Lactobacillus* spp.

Aerobic and Anaerobic Studies

Research performed during the 1970s indicated that mineralization of TNT and other nitrated munitions compounds did not occur to any significant extent (Carpenter et al., 1976). Although various system parameters were investigated, experiments were generally carried out under aerobic conditions.

Carpenter et al. (1976) investigated the microbial transformation of ^{14}C -Labeled 2,4,6-trinitrotoluene in an activated sludge system under aerobic conditions. The researchers detected less than 0.5% of the added ^{14}C as $^{14}\text{CO}_2$ in the head gas at the end of the experiment. This indicated that mineralization did not occur to any significant extent. Most of the ^{14}C was recovered from the water column and sediment bound in a polymeric precipitate which was resistant to further degradation.

McCormick et al. (1976) investigated the degradation of TNT and other nitroaromatic compounds under anaerobic conditions by cellular extracts and growing cultures of *Clostridium pasteurianum*, *Veillonella alcalescens*, *E. coli* and *Pseudomonas*. *E. coli* was also investigated under aerobic conditions.

The researchers concluded that *C. pasteurianum*, *V. alcalescens* and both the aerobically and anaerobically incubated *E. coli* produced nitro-reductases capable of catalyzing the transformation of one or more nitro groups to amino groups. Many other nitrated compounds investigated by the researchers were susceptible to reduction by the nitro-reductases of these microorganisms.

Nitroaromatic azoxy compounds were detected in growing cultures under aerobic conditions; however, no azoxy compounds were detected in

anaerobically incubated cultures. Additionally, the researchers noted the preferential reduction of the para nitro- group in the TNT molecule.

McCormick et al. (1981) studied RDX removal under aerobic and anaerobic conditions from nutrient broth cultures. Cultures were inoculated with activated sludge and dosed with either 50 or 100 ppm RDX. Aerobic systems were incubated at 30°C and anaerobic systems at 37°C.

RDX was completely removed from anaerobic systems after 4 days with subsequent formation of hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine, hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine, hexahydro-1,3,5-trinitroso-1,3,5-triazine, formaldehyde 1,1-dimethylhydrazine, 1,2-dimethylhydrazine and methanol.

The researchers recovered less than 1.5% of the initial ^{14}C dose from volatile material with the remainder tied up in organic compounds in the solution. No triazine derivatives beyond the nitroso- reduction were detected leading the researchers to conclude that reduction of the substituent groups beyond this point destabilized the molecule resulting in hydrolytic cleavage of the ring structure. No change in RDX concentration over the 4 day experimental period occurred under aerobic conditions leading researchers to conclude that any treatment system for wastewater containing RDX must include an anaerobic stage because of its aerobic recalcitrance.

Funk et al. (1993) examined the bioremediation of soils contaminated with TNT, RDX, and HMX using a microbial inoculum obtained from a dinoseb contaminated soil. The munitions contaminated soil was obtained from the US

Army munitions depot near Umatilla, Oregon.

Soil samples (4 g) were added to 500 mL Erlenmeyer wide-mouth flasks resulting in TNT, RDX and HMX concentrations of 120 ppm, 30 ppm and 3 ppm respectively. Under optimum anaerobic conditions, TNT was reduced to DANT within 10 days. The optimum pH for the reductive stage of TNT metabolism was found to be near neutral and the optimum temperature range was 25 - 37°C. Redox potential remained above 50 mV in systems receiving TNT until the ADNT metabolites were removed. The system oxidation/reduction potential then dropped to approximately -200 mV.

Under aerobic conditions, TNT was removed from the systems within 4 days; however, no intermediates were observed and a red-brown precipitate accumulated. The researchers concluded that the reductive stage of TNT metabolism had been observed in 24 days compared to 90 days which had been previously observed.

Shen et al. (1998) studied the fate of [^{14}C]-TNT and [^{14}C]-RDX in 100 mL microcosm soil bioslurries at concentrations of 1000 and 2000 mg/ kg respectively. Activated sewage sludge additions were investigated for their effect on ultimate mineralization. All microcosms were incubated at 28°C.

Aerobic conditions resulted in less than 0.5 % mineralization after 52 days. The addition of activated sludge resulted in a decrease in the amount of TNT mineralized possibly resulting from binding of metabolites to the organic matrix.

Under anaerobic conditions, numerous TNT metabolites including aminodinitrotoluene's and diaminonitrotoluene's were detected in soil systems

after 35 days; however, less than 1% mineralization was observed after 52 days of incubation. Soil systems receiving sludge additions had similar mineralization rates.

Under aerobic conditions, less than 1.9% mineralization of RDX was observed at 35 days. The addition of activated sludge only slightly increased this value to 2.2 %. Anaerobic conditions resulted in 15% mineralization of RDX after 52 days in the soil bioslurries. Addition of sewage sludge increased this value to 60%. Researchers also noted a slight inhibition of RDX mineralization in anaerobic systems which also received TNT.

Anaerobic Studies

Boopathy and Kulpa (1994) investigated 2,4,6-trinitrotoluene biotransformation by *Methanococcus* sp. (strain B) isolated from a lake sediment. *Methanococcus* sp. (strain B) is an anaerobic microorganism which reduces carbon dioxide during the oxidation of simple organic compounds with the subsequent production of methane and carbon dioxide.

Formate or hydrogen in a mineral salts medium served as the substrate and carbon dioxide was provided as an electron acceptor. The researchers observed the degradation of 100 ppm TNT within 40 to 60 days and the formation of 2,4-diamino-6-nitrotoluene (24DANT). The researchers observed no further transformation of 24DANT. Bottles receiving TNT as the sole carbon and energy source experienced no reduction in the initial TNT concentration of 100 ppm after 9 months.

The researchers concluded that the inability of *Methanococcus* sp. (strain B) to utilize TNT in the absence of other substrates indicated the cometabolic nature of TNT degradation by this particular microorganism. The researchers found that for this particular organism, the optimal pH range was 6.8 – 7.2 and the optimum temperature was in the mesophilic range at 30°C. The researchers recommended using a mixed culture anaerobic systems to optimize the rates of transformation and facilitate complete mineralization of various nitrated compounds.

Boopathy and Kulpa (1992) investigated TNT as a sole nitrogen source for *Desulfovibrio* sp. (B strain) isolated from an anaerobic digester. The bacterium used TNT as the sole nitrogen source and converted it to toluene through reductive deamination. The bacterium could also use nitrate, nitrite and ammonium as nitrogen sources. The researchers also demonstrated that nitrate, nitrite, and TNT could serve as electron acceptors in the absence of sulfate.

TNT was reduced from its initial concentration of 100 ppm to 0 ppm in approximately 8 days under nitrogen limiting conditions. Diaminonitrotoluene was identified as the major intermediate formed. When an additional source of ammonium was present, the *Desulfovibrio* species studied did not complete the reduction of diaminonitrotoluene to toluene.

After 45 days, 98 ppm toluene was detected in the systems; however, the bacterium was not capable of further transformations. The researchers concluded by suggesting that a diculture approach containing the *Desulfovibrio* species studied and a toluene degrading *Pseudomonas* spp.

Costa et al. (1996) and Boopathy et al. (1996) investigated the ability of *Desulfovibrio desulfuricans* (Strain A) to degrade TNT under various electron acceptor and substrate conditions. Under sulfate reducing conditions, 100 ppm TNT was transformed in 14 days with the formation of 4-amino-2,6-dinitrotoluene, 2-amino-4,6-dinitrotoluene, and 2,4-diamino-6-nitrotoluene. Under nitrate reducing conditions, butyric acid was formed by the microorganism.

The researchers noted that TNT transformation occurred only when an additional carbon source was present. The *D. desulfuricans* strain under investigation was able to use pyruvate, malate, ethanol, propanol, and butanol as substrates. The optimum temperature for growth was found to be 25 C and the optimum pH was 6.8

Ederer et al. (1997) examined 2,4,6-trinitrotoluene transformation by several *Clostridium bifermentans* species isolated from a munitions fed bioreactor, several related *Clostridium* species obtained from a culture collection, two enteric bacteria (*Escherichia coli* and *Salmonella typhimurium*) and three lactobacilli (*Lactobacillus casei*, *L. acidophilus*, and *L. lactis*). All experiments were carried out at 37°C under anaerobic conditions in brain heart infusion broth supplemented with 0.5% yeast extract.

All microorganisms studied were able to transform TNT to DANT. Three of the *Clostridium* species completely transformed 50 ppm TNT and byproducts in 12 hours. *L. casei* and *S. typhimurium* completely transformed TNT in 12 hours and transformed byproducts within 25 hours.

Clostridium species isolated from the munitions fed bioreactor did not display an increased resistance to TNT in terms of growth inhibition.

Transformation byproducts and similar rates additionally indicated the similarity of the degradation pathway by the *Clostridium* species. These results supported the researchers general belief that the ability to reduce TNT anaerobically is very common in nature.

Osmon and Klausmeier (1972) investigated the ability of microflora from various sources to degrade TNT and RDX on 1% glucose agar plates to which 100 ppm had been added. The effects of organic supplementation in the form of a 1% yeast extract and nitrogen supplementation as ammonium nitrate, ammonium chloride, or sodium nitrate were examined. TNT and RDX were also investigated for their ability to serve as sole nitrogen sources.

Sewage effluent, TNT loading facility effluent, a soil suspension, pond water and aquarium water were chosen as microbial sources. The agar plates were incubated for 2 to 4 days and then flooded with 20% NaOH to qualitatively determine TNT removal using the diethylaminoethanol method.

All systems that received both organic and nitrogen supplementation experienced greater than 94% reduction in TNT concentration in 6 days. Less than 6% TNT degradation was observed in systems that did not receive organic supplementation.

Ammonium nitrate, ammonium chloride and sodium nitrate were all found to be equally effective as nitrogen sources. Less than 11% degradation was observed in systems that received TNT as its sole nitrogen source. The

researchers observed no reduction in RDX concentration over this period under all system conditions examined. The researchers noted that the microorganisms involved appeared to be a *Pseudomonas* species based upon their production of a greenish-yellow diffusible pigment.

Toze et al. (1999) investigated the microbial degradation of TNT, RDX, nitrotoluenes and dinitrotoluenes in microcosms developed from sterilized production wastewater. The microcosms were constructed from 500 mL borosilicate bottles and buffered to a pH of 6.5. A 1% starchy potato waste was added as a carbon source.

Experimental microcosms were seeded with microorganisms isolated from the production wastewater and maintained for 12 months in an anaerobic defined salts medium with TNT and RDX as the only carbon source. Control reactors were left sterile.

The concentration of TNT decreased from 8.2 ppm to 2.0 ppm in the experimental reactors over 45 days with the formation of a small amount of 4-amino-2,6-dinitrotoluene. Decreases in the experimental reactors were significantly greater than the sterile control microcosms.

The researchers noted that most of this removal occurred in the first 20 days of incubation indicating the possible formation of an inhibitory compound. Significant reductions were also noted for 2-nitrotoluene, 3-nitrotoluene, 4-nitrotoluene, 2,4-dinitrotoluene, and 2,6-dinitrotoluene in experimental reactors versus the sterile controls. RDX concentrations decreased from 10.6 ppm to 6.8 ppm in the experimental reactors over 45 days which was a significantly greater

reduction than in the control systems.

Binks, Nicklin, and Bruce (1995) investigated the degradation of RDX by the bacterium *Stenotrophomonas maltophilia* isolated from a mixed microbial culture obtained from soil enrichments under aerobic and anoxic conditions. The bacterium was capable of using RDX as a sole nitrogen source; however, it could not use HMX as a sole nitrogen source. The researchers noted that the bacterium was only capable of degrading RDX when glucose was added to the systems indicating the cometabolic nature of the process.

Bradley et al. (1994) investigated the transformation of TNT and DNT by microorganisms collected from surface soils and aquifer materials at a munitions contaminated site. The researchers observed approximately 11% transformation of [14C]TNT to $^{14}\text{CO}_2$ over 35 days in soil systems and less than 1% transformation in the systems receiving aquifer materials. The lower transformation in the aquifer material was expected by the researchers because of the significantly lower organic content.

Abiotic Transformation

Gorontzy et al. (1996) investigated the abiotic reduction of aromatic nitro groups to the corresponding amino compounds by reducing agents such as cysteine, sulfide, titanium(III) or dithionite. The milder reducing agents represented by the first two compounds were found to reduce o-nitrophenol and 2,4-dinitrophenol when 1.5 mM sulfide was present in a phosphate buffer; however, p- and m-nitrophenol and p-nitrobenzoate were not reduced. The higher reduction potential of the second two compounds resulted in complete

reduction of all nitro groups to the corresponding amino compounds. Preuss and Rieger (1995) observed the abiotic reduction of TNT by sulfide and Heijman et al. (1996) reported the abiotic reduction of 4-chloronitrobenzene with Fe (II).

Hydrolysates of RDX and HMX

Alkaline Hydrolysis

High temperature alkaline hydrolysis rapidly decomposes RDX and HMX to simpler compounds by exposing them to a high temperature and high pH environment (Spontarelli et al., 1993). Heilmann et al. (1996) investigated alkaline hydrolysis of RDX and HMX with sodium hydroxide. Solution temperature ranged from 50°C to 80°C and pH was varied from 10 to 12. After completion of the hydrolysis reaction, the mixture was analyzed to determine its composition. The products of the alkaline hydrolysis of 1 mole of RDX are presented in Table 2-1.

The RDX decomposed much more rapidly than HMX under the same conditions. At a pH of 12 and a solution temperature of 80°C, alkaline hydrolysis of HMX is more than 99.9% complete after 100 minutes. Under the same conditions, RDX hydrolysis is complete in under 5 minutes.

Activated Sludge Treatment

Zoh and Stenstrom (1999) investigated the use of a denitrifying column to treat the waste products resulting from the alkaline hydrolysis of RDX and HMX. A 98 mL column was constructed and packed with 42 grams of cut silicon tubing. The column was initially inoculated with 1 mL of a mixed culture seed from

activated sewage sludge. The hydrolysate waste was synthetically generated based on the analyses in Table 2-1 provided by Heilmann et al. (1996).

Table 2-1. Products of alkaline hydrolysis of 1 mole RDX

Conc	Units	Compound
1.6	M	Nitrite (NO ₂ ⁻)
1.5	M	formate (HCOO ⁻)
0.10	M	acetate (CH ₃ COO ⁻)
1.1	M	formaldehyde (HCHO)
0.90	M	Ammonium (NH ₃)
1.1	M	nitrous oxide (N ₂ O)
0.34	M	nitrogen gas

Hydrolysate flow through the column had a mean retention time of three hours and resulted in the removal of greater than 90% of the nitrite and organic compounds present in the hydrolysate. The maximum nitrate nitrogen removal rate was 170 mg/L/day with the carbon sources present in the column and solution.

During oxidation of the hydrolysates, the researchers noted the production of alkalinity. In order to maintain the system pH in the ideal range of 7.0 to 7.5, the alkalinity must be neutralized or denitrification will cease. To neutralize the alkalinity generated by the oxidation of 1 mole of RDX, 4.33 moles of hydrochloric acid were required.

The researchers also investigated the systems ability to degrade RDX and HMX added to the reactors. The RDX and HMX were only degraded when

nitrate was present. The researchers noted that the presence of 5 mg/L of RDX and 3 mg/L of HMX inhibited carbon oxidation in the inflowing waste solution.

CHAPTER 3 MATERIALS AND METHODS

A standard operating procedure was developed for the startup and operation of 1.5 liter high anaerobic solids (HAS) reactors with and without TNT acclimation. Basic operating procedures were based on operational methodology as indicated by Lee (1992). A high solids anaerobic assay was developed to determine the rates of transformation of TNT, RDX and HMX by TNT acclimated and non-acclimated sludge.

Total gas production from biochemical methane potential (BMP) assays was measured to gauge system anaerobic microbial response during exposure to TNT, RDX, HMX, the untreated hydrolysates of RDX and HMX, and aerobically treated RDX and HMX hydrolysates.

Equipment and Materials

A Perkin Elmer series 200 solvent delivery pump with a Perkin Elmer diode array detector type 235C at a wavelength of 255 nm and a Merck KgaA LiChrospher RP18 column (5 μ m, 250mm x 4.6 mm) with a water:methanol carrier at a flow rate of 0.7 mL/min were used to quantify RDX, HMX, TNT, 4-amino-2,6-dinitrotoluene, 2-amino-4,6-dinitrotoluene, and an unidentified TNT transformation product. The water:methanol mixture was stepped from a 1:1 to 1:4 mixture on a volume:volume basis during sample analysis. This was followed

by flushing with 100 % methanol for 5 minutes and re-equilibration with the 1:1 mixture prior to the next analysis.

Anaerobic sludge reactors were constructed from 2 liter Nalgene bioculture bottles with built in magnetic stirrers as illustrated in Figure 3-1. The reactors were incubated with continuous stirring using Corning hotplate/stirrers.

Type J Cu/CuNi thermocouples wires were inserted into the reactors for temperature measurement. Wire ends were stripped, twisted together and coated with liquid electrical tape before installation in reactors to prevent corrosion. Temperature measurements were made using a Fischer Brand thermocouple meter.

Anaerobic reactor design is presented in Figure 3-1. Reactors were insulated with foil bubble wrap and sealed with silicon and aluminum tape to minimize temperature fluctuations and to help maintain a light and oxygen free environment. A gas collection system was constructed from 2 liter burets inverted and submerged in a 145 liter plastic trashcan to estimate gas generation rates as shown in Figure 3-1.

Two one-liter draw-and-fill activated sludge reactors were constructed using 2 liter glass bioculture bottles as shown in Figure 3-2. Reactors were stirred continuously using Corning stirrer/hotplates with 2.54 cm magnetic stirbars and aerated using a Pulsar aquarium air pump with a small bubblestone.

The BMP and high anaerobic solids assays were performed using 250 mL Wheaton crimp sealed serum bottles incubated in the dark at 35°C.

Mass determinations were performed on a Mettler AE 100 analytical balance with a precision of 0.0001 grams. Chemical Oxygen Demand (COD) digestions were performed using a wastewater COD block and quantified using a DR 4000u Hach Spectrophotometer. Nitrite levels were quantified using a DR 4000u Hach Spectrophotometer.

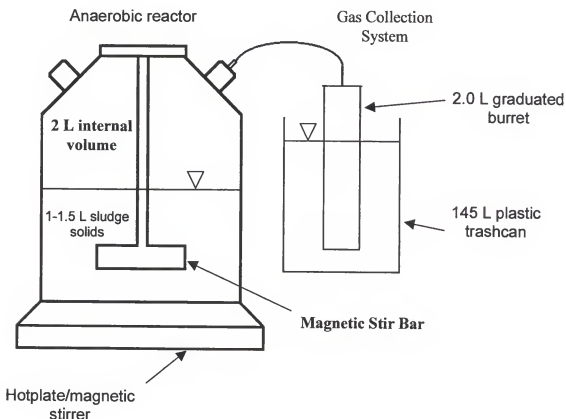


Figure 3-1. Anaerobic reactor and gas collection system cross-section.

Reagents and Standards

A HPLC grade methanol was used for extractions and for HPLC analysis. Standards for 2,4,6-trinitrotoluene, 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, and octahydro-1,3,5,7-

tetranitro-1,3,5,7-tetrazocine were obtained from ChemService, New Jersey. Standards for 2,4-diamino-6-nitrotoluene, 2,6-diamino-4-nitrotoluene were obtained from USA Scientific, California.

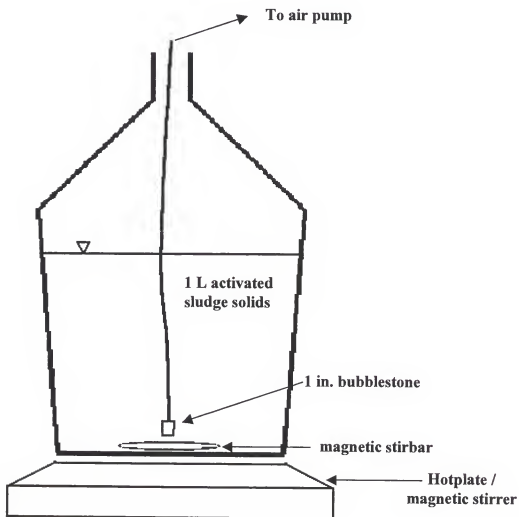


Figure 3-2. Activated sludge draw-and-fill reactor cross-section.

TNT used for preparing stock solutions was purchased from ChemService, NJ. The RDX and HMX were provided by Los Alamos National Laboratory. A 10,000 ppm stock solution was prepared for each of the three

explosive compounds by adding 1000.00 mg of explosive to 100.0 mL of acetonitrile. The solutions were stored at 3°C and warmed to 35°C before use.

The COD samples were prepared for analysis using Hach COD tubes (0 to 1500 ppm). Samples were prepared for nitrite analysis using Hach Nitrite Test-n-tubes (0 to 0.50 ppm).

Anaerobic Reactor Setup and Operation

Six 1 liter anaerobic reactor's described in the previous section and presented graphically in Figure 3-1 were set up and operated to produce a sludge inoculum for the biochemical methane potential (BMP) and high anaerobic solids (HAS) assays. The reactors were initially charged with activated sludge and horse manure in a 1:1.5 ratio based on dry solids content. Reactors were incubated at 35°C with continuous stirring.

Twenty liters of activated sludge were obtained from the waste activated sludge (WAS) line at the University of Florida Wastewater Reclamation Facility (UFWRF) on the campus of the University of Florida, Gainesville, Florida. The treatment plant receives wastewater from a variety of sources including student dorms, public restrooms, research laboratories, medical facilities, and livestock management operations. Activated sludge properties are presented in Appendix G.

The sludge blanket was allowed to settle to one-third of the initial volume and the supernatant was siphoned off. Triplicate samples of the settled sludge were collected for total and volatile solids analysis.

One liter of settled sludge was added to each high anaerobic solids reactors. Additionally, 50 grams of fresh horse manure was added to each of the 1 liter reactors. The horse manure was collected from a 14-year-old gelded quarterhorse that was fed one quart of 12 percent sweet feed daily and permitted to graze on Bahai and native grasses on a farm in Alachua County, Florida. The manure was added to the reactors within 60 minutes of generation.

The reactors were incubated at 35°C with continuous stirring. Stirring speed was maximized while minimizing the frequency at which the stir bar would begin skipping and stirring would cease. The maximum stirring frequency was found to be 20 rotations per minute (RPM) which corresponded to a stirring level setting of 2 out of 10 on the Corning hot plate/stirrer. Visual inspection indicated that this stirring speed was sufficient to maintain reactor solids in suspension. Three reactors were randomly selected for TNT acclimation. Reactors received a 50.0 mg dose of TNT every 14 days for a total of 6 doses per reactor.

Activated Sludge Reactor Setup and Operation

The pretreatment of RDX and HMX hydrolysates prior to BMP analysis was investigated in activated sludge systems for the purpose of denitrification. The hydrolysates were prepared as indicated in Appendix E by Los Alamos National Laboratory personnel.

The 1 liter draw-and-fill activated sludge systems were set up for treatment of the hydrolysates as shown in Figure 3-2. The rate of RDX and HMX hydrolysate additions and dilution were designed based on various hydrolysate properties including nitrite concentration, chloride concentration and biological

oxygen demand (BOD). Hydrolysate properties are presented in Table 4-1.

Dosing and dilution rates are presented in Table 4-2 and design calculations are presented in Appendix A.

Activated sludge reactors were charged with sludge from the WAS line at UFWWTP. The activated sludge reactors were stirred continuously and aerated for 30 minutes every 6 hours during research investigations for a total of 2 hours per 24 hour period.

The RDX and HMX activated sludge reactors were dosed repeatedly with either RDX or HMX hydrolysate. The system received no organic carbon inputs other than that supplied by the hydrolysate additions. The COD measurements were made at the beginning and end of experiments to calculate changes in total system COD. Hourly samples were collected from the reactors following dosing for nitrite analysis. System pH was measured at regular intervals and corrected to pH 8.2 using concentrated hydrochloric acid.

Biochemical Methane Potential Assays

A modified biochemical methane potential (BMP) assay methodology was employed to compare the effects of the treatments (Table 3-2) on total gas production as a function of time. Eight treatments were applied to the systems using a standard anaerobic sludge (Table 3-2). Additionally, TNT, RDX, HMX, and an equal mass mixture of the three were investigated using the TNT acclimated sludge for statistical comparison (Table 3-2).

Wheaton 250 mL serum bottles received 0.120 g of micronized cellulose as the standard substrate for the experiments. The bottles were then dosed with

either 0.120 g of the explosive compounds under investigation or a volume of hydrolysate equivalent to 0.120 g of the parent compound before filling with anaerobic inoculum.

Table 3-1. Treatments applied to BMP assays receiving non-acclimated and TNT acclimated sludge solids.

Control Treatments - Standard non-acclimated anaerobic sludge

1. TNT
2. RDX
3. HMX
4. Equal mass mixture of TNT, RDX, and HMX
5. Aerobically pretreated RDX hydrolysate*
6. Aerobically pretreated HMX hydrolysate*
7. Raw RDX hydrolysate**
8. Raw HMX hydrolysate**

Experimental Treatments - TNT acclimated sludge

1. TNT
2. RDX
3. HMX
4. Equal mass mixture of TNT, RDX, and HMX.

* Aerobically pretreated hydrolysates were denitrified using activated sludge draw-and-fill reactors.

** Raw hydrolysates received no biological pretreatment.

The anaerobic inoculum was prepared by mixing the desired sludge (unacclimated or TNT acclimated) with a standard media solution in a 1:4 ratio based on total volume. The standard media was prepared from standard concentrated stock solutions as described by Owen et al. (1979) and presented in Appendix G.

The standard media was prepared in a 2 liter Pyrex flask sealed using a No. 9 rubber stopper with two ports to permit flushing with 100% nitrogen mixture during preparation. Constant stirring during preparation was accomplished using a Corning hotplate/stirrer with a 2.54 cm magnetic stir bar.

The 250 mL bottles which had previously received substrate and investigational compounds were swept with nitrogen gas for 20 seconds before and after the addition of 250 mL of anaerobic inoculum to each. The bottles were filled with inoculum in random order, flushed with nitrogen for 20 seconds and crimp sealed. The bottles were stored inverted in the dark at 35°C.

Gas production was monitored at regular intervals to determine generation rates. Data was examined for lags in gas production and differences in the total quantities of gas produced. Gas generation rates were compared between treatments and sludge types.

High Anaerobic Solids Assay

A high solids anaerobic (HAS) assay was developed to determine the transformation rates of the compounds under investigation in an environment with a very low electronegative potential (<-300 mV) and microbial solids concentrations greater than 2% total solids. Assays were carried out in Wheaton 250 mL serum bottles filled with 100 mL sludge solids and incubated in the dark at 35°C.

Four treatments were applied to each sludge type as presented in Table 3-3. The three explosive compounds under investigation (TNT, RDX, and HMX) were applied to the anaerobic systems each at a level of 100 ppm. An

equalparts mixture of TNT, RDX, and HMX at a total concentration of 100 ppm constituted the fourth treatment. The four treatments for each sludge type were run in triplicate.

Table 3-3. Treatment schedule for high anaerobic solids assays.

Experimental Sludge

1. TNT – 100 ppm
2. RDX – 100 ppm
3. HMX – 100 ppm
4. Mix (TNT, RDX, and HMX) – 33 ppm each

Control Sludge

1. TNT – 100 ppm
 2. RDX – 100 ppm
 3. HMX – 100 ppm
 4. Mix (TNT, RDX, and HMX) – 33 ppm each
-

Approximately 750 mL of sludge was removed from each of the 1.5 liter anaerobic high solids reactors. Ninety-nine milliliters of sludge was added to each of six 250 mL Wheaton assay bottles. The bottles were flushed with nitrogen for 20 seconds and immediately crimp sealed.

One milliliter of the appropriate stock solution was injected into one of the six high anaerobic solids assay bottles previously filled from each reactor. The particular bottle injected into within each reactor subgroup was chosen randomly. Bottles were stored inverted at 35°C in a dark incubator.

Sample Collection and Analysis

Total solids (TS) and volatile solids (VS) determinations were made using methods 209.A and 209.D in Standard Methods for Wastewater Analysis (1998). Triplicate samples were collected for solids analysis from the anaerobic and activated sludge reactors periodically and prior to sludge usage in either BMP or high solids assays.

The RDX and HMX hydrolysates were analyzed prior to biological treatment operations. Ion chromatography was used for the initial and final quantification of nitrate, nitrite, and chloride. Samples were diluted 400:1 with distilled deionized water and filtered using a Whatman 0.47 μm filter before analysis.

Samples for nitrite analysis were prepared by adding 5 mL of the filtered and diluted sample to a Hach Nitrite (0 to 0.50 ppm) Test-N-Tube vial and quantified using a Hach DR 4000 Spectrophotometer. Nitrite standards at 0, 0.10, 0.25, and 0.5 ppm NO_2^- were run daily to confirm machine calibration.

COD samples were prepared by adding 3 mL's of the filtered and diluted sample to a Hach COD tube (0 to 1500 ppm). Samples were filtered using a 0.47 μm glass syringe filter after dilution. Samples were digested for 2 hours using a COD digestion block. Measurements were made using a DR 4000 spectrophotometer. COD standards at 0, 500, 1000, and 1500 ppm were run to confirm machine calibration.

Samples were withdrawn from the high anaerobic solids (HAS) assay bottles at predetermined intervals for quantification of compounds and

byproducts under investigation.

Concentrations of the parent explosive compounds in addition to various byproducts were quantified using high pressure liquid chromatography with UV detection at 254 nm and a 7 point standard curve.

Sampling, extraction and analysis procedures were adapted from EPA Method 8330A: Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC). Sampling and extraction procedures are presented in Table 3-4 below.

A mixed standard solution at the concentrations shown in Table 3-3 was prepared in acetonitrile. External standards at 50, 25, 12.5, 6.25, 3.125, 1.625, and 0.81 ppm were used for quantification of 2,4,6-trinitrotoluene (TNT), 2-amino-4,6-dinitrotoluene (2ADNT), 4-amino-2,6-dinitrotoluene (4ADNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetraazocine (HMX). One midrange sample was run approximately every 20 samples.

Two milliliter aliquots were withdrawn from the high anaerobic solids assay bottles and transferred to sterile 10 mL test tubes. Thirty microliters of concentrated HCL was immediately added to the sample. This was followed by the addition of 2 mL of HPLC grade methanol and 20 μ L of acetonitrile to enhance explosive recovery.

Samples were mixed for 30 minutes and then centrifuged for 30 minutes at 7000 x g. The supernatant was pipetted off and filtered using a 0.45 μ m glass fiber syringe filter with the first 1.0 mL discarded. The filtrate was then stored in a

2 mL amber glass vial with PTFE cap at 3°C for later analysis. Holding times for samples were the same as those for semivolatile organics (method 8330 A).

Table 3-4. Sampling and extraction procedure for high anaerobic solids assays.

-
1. Withdraw 2 mL aliquot
 2. Transfer to 10 mL test tube
 3. Add 30 μ L conc. HCl
 4. Mix for 1 minute.
 5. Add 2 mL HPLC grade methanol.
 6. Add 20 μ L acetonitrile.
 7. Mix for 30 minutes.
 8. Centrifuge for 30 minutes at 7000 x g.
 9. Pipet off supernatant
 10. Filter using a 0.45 μ m glass fiber syringe filter
 11. Discarding the first 1.0 mL of filtrate.
 12. Store filtrate in 2 mL amber vial with PTFE cap at 3°C.
-

Data Analysis and Modeling

A linear model as presented by Ott (1984) was used to investigate data trends present in the BMP and high solids data. The linear model is presented below:

$$Y = \beta_0 + \beta_1 x_1 + e \quad \text{Eq. 3-1}$$

where: $\beta_0 = y$ – intercept for regressed line

β_1 = Slope of the regressed line

x_1 = Parameter of interest

e = residual error

Explosive transformation and gas generation rates were compared using the linear model with the addition of a qualitative factor. The hypothesis that the

slopes of the two lines were different was investigated.

A dummy variable was assigned to the qualitative factor and given a value of 0 or 1 depending on whether the data set was for the control or experimental sludges respectively. The mathematical model was then used to determine if significant differences exist between the slopes of the two lines under comparison. The model is presented in Eq. 3-2 below.

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1 x_2 \quad \text{Eq. 3-2}$$

where: Y = waste characteristic of interest

x_1 = time

$x_2 = \{1 \text{ if TNT acclimated sludge solids}$

$\{0 \text{ if non-acclimated sludge solids}$

$\beta_0 = y$ – intercept for non-acclimated sludge solids

β_1 = slope of line for TNT acclimated sludge solids

β_2 = difference in y-intercepts of the two lines

β_3 = difference between the slopes of the two lines

Substituting for x_2 yields the expected equations for the two regression lines as shown in Eq. 3-3 and Eq. 3-4 below.

$$Y = \beta_0 + \beta_1 x_1 \quad \text{Eq. 3-3}$$

$$Y = (\beta_0 + \beta_2) + (\beta_1 + \beta_3) x_1 \quad \text{Eq. 3-4}$$

The model presented in Eq. 3-4 allows analysis of the data as a single data set. Additionally, a reduced model was run with $\beta_3 = 0$. Comparison of the full model output with that of the reduced model permits analysis of the change in the residual errors for the regressions to determine if a significant difference

between the data sets exists. A significant difference would indicate that the two data sets have different slopes, implying a rate difference exists between the acclimated and control sludges for the compound under investigation.

Minitab statistical software was used to investigate the data in both the reduced and full models. The reduction in the sum of squares error attributed to x_1x_2 was calculated using Eq. 3-5 below:

$$SS_{\text{drop}} = SSE_2 - SSE_1 \quad \text{Eq. 3-5}$$

where

SSE_2 = error sum of squares for the reduced model.

SSE_1 = error sum of squares for the full model.

Significance was determined by calculation of an F value for the change using Eq. 3-6 below. This F value was compared to the value from an F distribution table for the correct number of degrees of freedom for the drop with $\alpha = 0.05$. If the observed F value exceeded the table, then H_0 was rejected and the conclusion was reached that the two slopes were different.

$$F = MS_{\text{drop}} / MSE_1 \quad \text{Eq. 3-6}$$

where

MS_{drop} = Drop in mean square error between reduced and full models.

MSE_1 = Mean square error for full model

Quality Assurance and Laboratory Error Analysis

Research investigations and experiments were designed with quality assurance (QA) goals in mind to insure that the data generated would be

statistically valid. Error and completeness for laboratory procedures were calculated as indicated below.

Laboratory error was evaluated for various lab procedures using a pooled variance analysis. The variance for replicate measurements was determined for a particular sample analysis. The pooled variance and completeness are calculated using Eq. 3-7 and Eq. 3-8 below.

$$S_p^2 = \frac{\sum S_i^2 (n_i - 1)}{(\sum n_i) - N} \quad \text{Eq. 3-7}$$

where: S_p^2 = pooled variance

S_i^2 = sample variance of i th sample

n_i = number of measurements in the i th sample

N = total number of samples in analysis

$$C = (100\%)(V/T) \quad \text{Eq. 3-8}$$

where: C = Completeness (%)

V = total number of valid measurements

T = total number of measurements

CHAPTER 4 RESULTS AND DISCUSSION

Activated Sludge Treatment of RDX and HMX Hydrolysates

Treatment of RDX and HMX hydrolysates was investigated in draw-and-fill activated sludge systems to address the high nitrite levels in the hydrolysate wastes. No additional COD was provided other than that present in the hydrolysates.

RDX and HMX hydrolysate properties were investigated and are presented in Table 4-1. The RDX and HMX hydrolysates had slightly basic pH's of 7.80 and 7.84 respectively and TDS levels of 29290 mg/L and 24630 mg/L, respectively. A significant portion of the total dissolved solids (TDS) in the hydrolysates can be accounted for by high waste nitrite levels and salts from the neutralization process. The nitrite concentration of 23350 mg/L as NO_2^- accounted for 79.7% of the TDS in the RDX hydrolysate and the nitrite concentration of 16,060 mg/L as NO_2^- accounted for 65.2% of the TDS in the HMX hydrolysate. High hydrolysate chloride levels resulted from neutralization of excess sodium hydroxide with hydrochloric acid prior to shipping.

Table 4-2 presents dosing data for the two draw-and-fill activated sludge reactors. The reactors were dosed daily with either 22.8 mL of RDX hydrolysate (2.28% by volume) or 33.2 mL of HMX hydrolysate (3.32%). Doses were based

on a target nitrite dose of 500 ppm as NO_2^- . Hydrolysates were diluted 1:1 before addition to keep chloride levels below 6000 ppm based on HMX chloride levels.

The generation of alkalinity during nitrite reduction required the addition of hydrochloric acid to maintain the reactors in the desired pH range of 8 to 9. This required 29.5 μL of concentrated HCL per mL of HMX hydrolysate and 44.2 μL of HCl per milliliter of RDX hydrolysate.

Repeat dosing data for the activated sludge systems are presented graphically in Figures 4-1 and 4-2. Raw data values are presented in Appendix C. Results are summarized below in Table 4-3.

Both reactor systems repeatedly denitrified greater than 99% of the added nitrite within 24 hours of dosing. Chemical oxygen demand (COD) reduction was 67.2% and 65.5% for the systems receiving RDX and HMX hydrolysates respectively over the length of the experiment.

Analysis of hydrolysates prior to treatment indicated the presence of 0.004% by mass HMX residue and no detectible RDX residue in their respective hydrolysates. No explosive residues were detected within the treatment systems 5 days after the last hydrolysate additions.

Nitrite reduction rate curves and equations for RDX and HMX hydrolysates are presented in Figure 4-3 and Figure 4-4. The mathematical model developed in Chapter 3 for regression slope analysis was used to determine the effect of hydrolysate type on nitrite reduction rates. Analysis indicated a greater rate of nitrite reduction ($F_{1,11} = 126.3 > F_{1,11,0.05} = 4.84$) in systems receiving HMX hydrolysate compared to systems dosed with RDX hydrolysate.

Theoretical calculations of the hydrolyzed quantities of RDX and HMX based on waste nitrite levels were performed to determine the treatment potential of the activated sludge units. The RDX hydrolysate contained 37,600 mg/L hydrolyzed RDX and the HMX hydrolysate contained 25,900 mg/L hydrolyzed HMX.

Table 4-1. Properties of RDX and HMX hydrolysates

Parameter	Units	RDX	HMX
pH	Std. Units	7.80	7.84
Total dissolved solids	Ppm	29290	24630
Chemical Oxygen Demand	mg/L O ₂	38440	32200
Biological Oxygen Demand*	mg/L O ₂	10100	9100
Nitrite	mg/L NO ₂ ⁻	23350	16060
Nitrate	mg/L NO ₃ ⁻	1086	1092
Chloride	mg/L Cl ⁻	4856	7478
Explosive Residual	%	<0.0001	0.004

* Determination made by Kanapaha Waste Water Treatment Plant

Table 4-2. Dosing data for RDX and HMX hydrolysates in activated sludge reactors.

Parameter	Units	RDX Reactor	HMX Reactor
Volume	ml	1000	1000
Total Solids	%	0.75	0.72
Hydrolysate per dose	ml	22.8	33.2
Hydrolysate additions	#	17	19

Table 4-3. Treatment results for RDX and HMX hydrolysates in activated sludge systems.

Parameter	Units	RDX Reactor	HMX Reactor
Total Nitrite Addition	mg/L NO_2^-	7950	9470
Final Nitrite Concentration	mg/L NO_2^-	1.7	0.9
Nitrite Removal	%	99.9	99.9
Maximum COD	mg/L O_2	10210	11350
Final COD	mg/L O_2	3920	4510
COD Removal	%	67.2	65.3
Explosive Residual	%	<0.0001	<0.0001

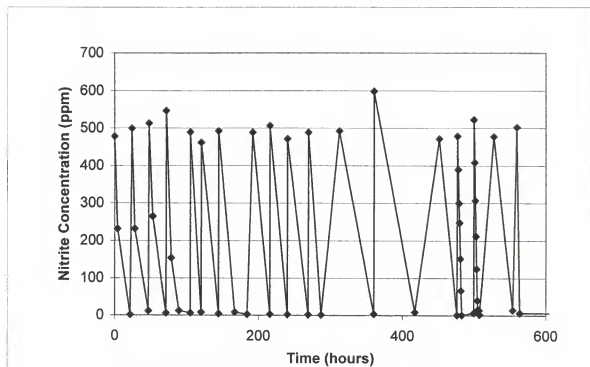


Figure 4-1. Repeat dosing of a draw-and-fill activated sludge reactor with RDX hydrolysate showing nitrite concentration.

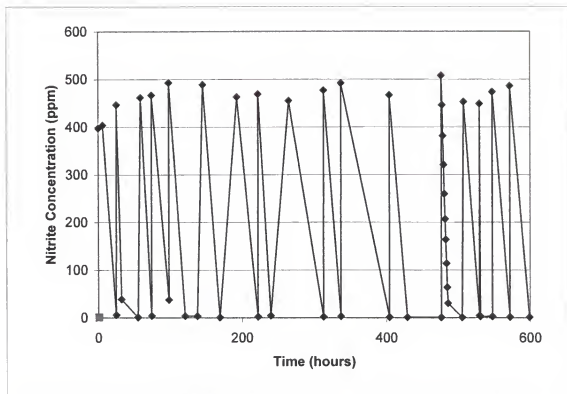


Figure 4-2. Repeat dosing of a draw-and-fill activated sludge reactor with HMX hydrolysate showing nitrite concentration.

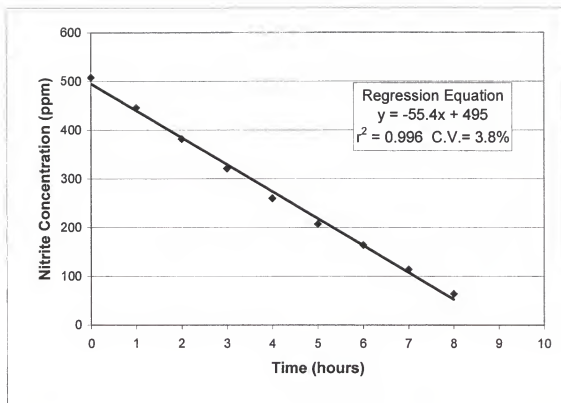


Figure 4-3. Nitrite concentration versus time in a draw-and-fill activated sludge reactor receiving RDX hydrolysate.

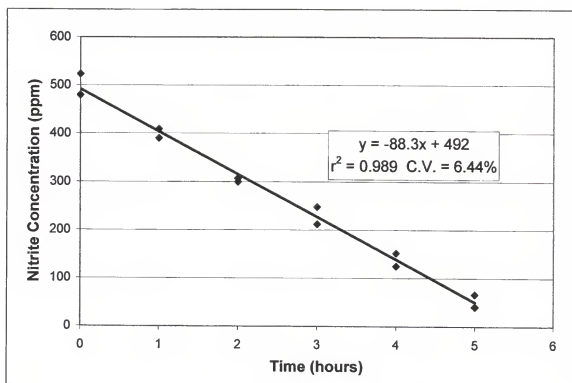


Figure 4-4. Nitrite concentration versus time in a draw-and-fill activated sludge reactor receiving HMX hydrolysate.

Modified Biochemical Methane Potential Assay

A modified BMP assay was performed with measurement of total gas over a 90 day period. Gas generation results are presented in Figures 4-5 through 4-10. Raw data values are included in Appendix D and statistical analyses using Minitab software are presented in Appendix K.

Analysis of Control Treatment

Gas production curves from control treatments for non-acclimated and TNT acclimated sludge solids are presented in Figure 4-5. Non-acclimated sludge solids reactors receiving no explosive additions (control treatment) produced an average of 276.5 cm^3 ($\sigma = 3.77$) of gas in 90 days with 90% ($t_{90\%}$) produced in 18.1 days. Reactors receiving the TNT acclimated sludge solids produced an average of 272.5 cm^3 ($\sigma = 3.97$) of gas in 90 days with 90% produced in the first 18.9 days. Data analysis results are summarized in Table 4-4.

TNT acclimated and non-acclimated sludge solids were compared within the control treatment using a two sample t-test assuming equal variances. Statistical analysis yielded a t test statistic of 0.63 ($P = 0.56$). This was less than the t-table value of 2.13 ($\alpha = 0.05$ and $df = 4$) (Kleinbaum, Kupper and Muller, 1988); therefore, the null hypothesis of equal means could not be rejected indicating that no differences existed between sludge solids types. Results are summarized in Table 4-4 below.

Statistical analysis of $t_{90\%}$ for the TNT acclimated and non-acclimated sludge solids using a two-sample t-test assuming equal variances generated a

test statistic of 1.74. This was less than the table value of 2.13 ($\alpha = 0.05$, $df = 4$); therefore, the null hypothesis of equal means could not be rejected indicating that no difference existed for the time period to 90% total gas production between sludge types. Results are summarized in Table 4-4.

Table 4-4. Summary of results for BMP assays.

Treatment	Sludge Type	Cumulative Gas Production (cm ³)	Standard Deviation (σ)	t _{90%} (days)	Standard Deviation (σ)
Control	TNT acclimated	274.5	3.97	18.1	0.59
Control	unacclimated	276.5	3.77	18.9	0.49
Control	Combined data set	275.5	3.63	18.6	0.65
TNT	TNT acclimated	60.5	3.77	NA	NA
TNT	unacclimated	62.2	4.54	NA	NA
TNT	Combined data set	61.3	3.84	NA	NA
RDX	TNT acclimated	268.7	6.17	75.4	2.01
RDX	un-acclimated	270.3	3.51	70.6	1.40
RDX	Combined data set	269.5	4.58	73.0	3.08
HMX	TNT acclimated	273.7	5.62	48.0	0.80
HMX	unacclimated	275.8	4.54	47.4	0.73
HMX	Combined data set	274.8	4.72	47.7	0.75
Treated RDX hydrolysate	unacclimated	277.8	2.57	18.0	2.28
Raw RDX hydrolysate	unacclimated	286.5	4.09	45.5	0.43
Treated HMX hydrolysate	unacclimated	281.2	4.75	17.8	1.20
Raw HMX hydrolysate	unacclimated	286.8	3.06	36.4	1.70

Table 4-5. Comparison of BMP assay results for TNT acclimated versus non-acclimated sludge solids receiving control, TNT, RDX, and HMX treatments.

Treatment	Comparison	Parameter	α	df	t-test value	$t_{0.05}$	P
Control	TNT acclimated vs. non-acclimated	total gas	0.05	4	0.63	2.13	0.56
		$t_{90\%}$	0.05	4	1.74	2.13	0.16
TNT	TNT acclimated vs. non-acclimated	total gas	0.05	4	0.49	2.13	0.65
		$t_{90\%}$	0.05	4	3.44	2.13	0.03
RDX	TNT acclimated vs. non-acclimated	total gas	0.05	4	0.41	2.13	0.71
		$t_{90\%}$	0.05	4	0.05	2.13	0.63
HMX	TNT acclimated vs. non-acclimated	total gas	0.05	4	0.05	2.13	0.63
		$t_{90\%}$	0.05	4	0.88	2.13	0.43

Table 4-6. Comparison of treatment effects for BMP assays.

Treatments Compared	Parameter	α	df	t-test value	$t_{0.05}$	P
TNT (combined data) vs Control (combined data)	total gas	0.05	10	99.2	1.81	<0.0001
RDX (combined data) vs control (combined data)	total gas	0.05	10	2.51	1.81	0.031
	$t_{90\%}$	0.05	10	42.4	1.81	<0.0001
HMX (combined data) vs control (combined data)	total gas	0.05	10	0.31	1.81	0.76
	$t_{90\%}$	0.05	10	42.4	1.81	<0.0001
RDX hydrolysate (treated) vs RDX hyd. (untreated)	total gas	0.05	4	3.11	2.13	0.036
	$t_{90\%}$	0.05	4	20.6	2.13	<0.0001
RDX hydrolysate (treated) vs Control	total gas	0.05	7	0.98	1.90	0.36
	$t_{90\%}$	0.05	7	0.41	1.90	0.70
RDX hydrolysate (untreated) vs control	total gas	0.05	7	4.13	1.90	0.004
	$T_{90\%}$	0.05	7	11.8	1.90	<0.0001
HMX hydrolysate (treated) vs HMX hyd. (untreated)	total gas	0.05	4	1.74	2.13	0.16
	$T_{90\%}$	0.05	4	15.5	2.13	0.0001
HMX hydrolysate (treated) vs Control	total gas	0.05	7	2.01	1.90	0.084
	$T_{90\%}$	0.05	7	1.06	1.90	0.32
HMX hydrolysate (untreated) vs control	total gas	0.05	7	4.61	1.90	0.0025
	$T_{90\%}$	0.05	7	25.9	1.90	<0.0001

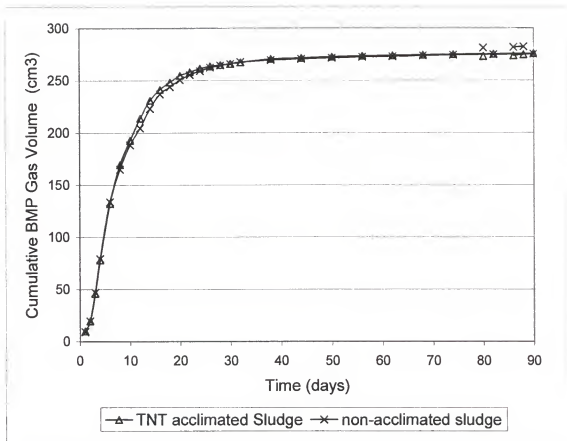


Figure 4-5. Cumulative gas volume versus time for control treatments of BMP assays with TNT acclimated or non-acclimated sludge solids.

Analysis of TNT Treatment

Cumulative gas volume versus time for BMP assays receiving the TNT treatment and either TNT acclimated and non-acclimated sludge solids are presented in Figure 4-6. The non-acclimated sludge produced an average of 62.2 cm^3 of total gas in 90 days. This volume of gas comprised only 23% of the expected total of 274.5 cm^3 generated by control BMP reactors. Reactors receiving the TNT-acclimated sludge produced an average of 60.5 cm^3 of total gas in 90 days comprising only 22% of the expected total of 276.5 cm^3 .

Comparison of total gas produced between sludge types at 90 days using a two sample t-test assuming equal variances yielded a test statistic of 0.49. This was less than the table value of 2.13 ($\alpha = 0.05$, $df = 4$); therefore, the null hypothesis could not be rejected leading to the conclusion that no statistically significant difference was evident between sludge types.

Data sets for the two sludge types receiving the TNT treatment were combined for comparison with the combined control data set. Comparison using a t-test under the parameters discussed above yielded a test statistic of 99.2. This exceeded the table value of 1.81 ($\alpha = 0.05$) and the null hypothesis was rejected. It was concluded that a significant difference existed between the means at 90 days. It was concluded that TNT additions did have an inhibitory effect on the rate of gas production in the systems.

Analysis of RDX Treatment

Cumulative gas volume versus time for BMP assays receiving the RDX treatment and either TNT acclimated or non-acclimated sludge solids are presented in Figure 4-7. BMP assays receiving non-acclimated sludge solids produced an average of 270.3 cm^3 ($\sigma = 3.51$) of total gas in 90 days with 90% produced in 70.6 days ($\sigma = 1.40$). BMP assays receiving TNT acclimated sludge solids produced an average of 268.7 cm^3 ($\sigma = 6.17$) of total gas in 90 days with 90% produced in the first 75.4 days ($\sigma = 2.01$).

Comparison of total gas produced between sludge types using a two sample t-test assuming equal variances yielded a test statistic of 0.41 ($P = 0.71$). This was less than the table value of 2.13 ($\alpha = 0.05$, $df = 4$); therefore, the null

hypothesis of equal means could not be rejected indicating that differences were not present in the data set.

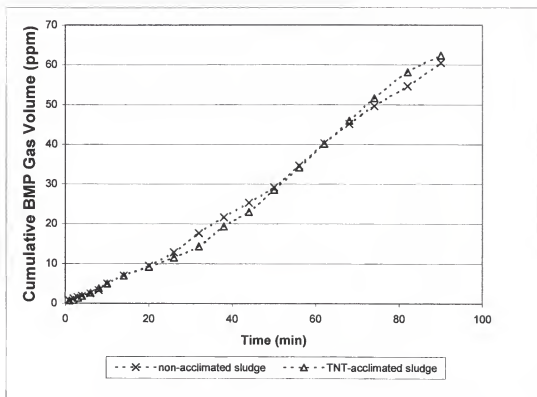


Figure 4-6. Cumulative gas volume versus time for TNT treatments of BMP assays with TNT acclimated or non-acclimated sludge solids.

Analysis of the time required to reach 90% of total gas generation ($t_{90\%}$) for the two sludge types using the same procedure generated a t-test statistic of 3.44 which was greater than the table value of 2.13 ($df = 4$, $\alpha = 0.05$). The null hypothesis of equal mean production time's was rejected and it was concluded that a statistically significant difference existed between the TNT acclimated and non-acclimated sludge types.

Data sets for the two sludge types receiving the RDX treatment were combined for comparison with the combined control data set from the control BMP assays. The combined RDX data set had a mean gas generation volume of 269.5 cm^3 ($\sigma = 4.58$) with 90% produced in the first 73.0 days ($\sigma = 4.58$). Comparison of total gas produced at 90 days using a t test under the parameters discussed above yielded a test statistic of 2.51 which exceeded the table value of 1.81 ($\alpha = 0.05$, $df = 10$). The null hypothesis was rejected and it was concluded that a statistically significant difference existed in the total mean gas generation between the RDX and control treatments.

Comparison of the time to produce 90% of the total gas evolved using a t-test yielded a test statistic of 42.4. This exceeded the table value of 1.81 ($\alpha = 0.05$, $df = 10$) and the null hypothesis was rejected. It was concluded that the time period to achieve 90% of the total gas production was different between RDX and control (no explosive additions) treatments.

Analysis of HMX Treatment

Cumulative gas volume versus time for BMP assays receiving the HMX treatment and either TNT acclimated or non-acclimated sludge solids are presented in Figure 4-8. BMP assays receiving unacclimated sludge produced an average of 275.8 cm^3 ($\sigma = 4.54$) of total gas in 90 days with 90% produced in 47.5 days ($\sigma = 0.727$). BMP assays receiving TNT acclimated sludge produced an average of 273.7 cm^3 ($\sigma = 5.62$) of total gas in 90 days with 90% produced in the first 48.0 days ($\sigma = 0.803$).

Comparison of total gas produced between sludge types using a two sample t-test assuming equal variances yielded a test statistic of 0.52 ($P = 0.63$). This was less than the table value of 2.13 ($\alpha = 0.05$, $df = 4$); therefore, the null hypothesis could not be rejected and it could not be concluded that a significant difference exists between the sludge types.

Analysis of the time required to reach 90% of total gas generation for the two sludge types using the same procedure generated a t-test statistic of 0.88 ($P = 0.43$). This was less than the table value of 2.13. The null hypothesis could not be rejected and it could not be concluded that a difference existed between the sludge types.

Data sets for the two sludge types receiving the HMX treatment were combined for comparison with the combined control data set. Comparison of total gas produced at 90 days using a two-sample t test assuming equal variances yielded a test statistic of 0.31 which was less than the table value of 2.13. The null hypothesis was not rejected and it could not be that a difference existed between the control (no explosive additions) and HMX treatments.

Comparison of the time to produce 90% of the total gas evolved in 90 days using a t-test a test statistic of 72.3. This exceeded the table value of 1.81 ($\alpha = 0.05$, $df = 10$) and the null hypothesis of equal mean's was rejected. It was concluded that a significant difference existed between the control and HMX treatments. Using a one-tailed analysis it was concluded that HMX additions resulted in a statistically significant longer period of time to reach 90% of total gas production ($P < 0.0001$) compared to controls.

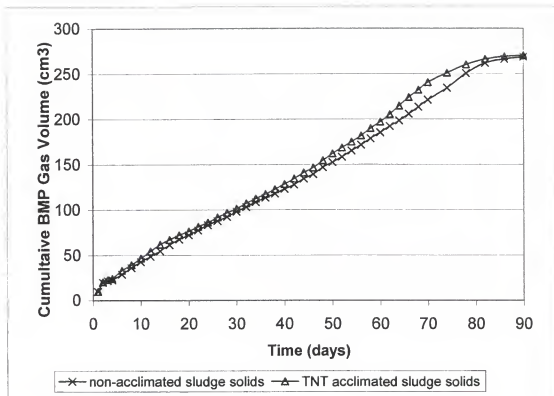


Figure 4-7. Cumulative gas volume versus time for RDX treatment of BMP assays receiving TNT acclimated or non-acclimated sludge solids.

Analysis of Raw and Denitrified HMX Hydrolysate Treatments

Cumulative BMP Gas production results from the aerobically treated and untreated HMX hydrolysates are presented in Figure 4-9 below. Treated HMX hydrolysate produced an average of 281.2 cm^3 of gas in 90 days with 90% of that occurring in 17.8 days. Untreated HMX hydrolysate produced an average of 286.8 cm^3 ($\sigma = 2.84$) with 90% of that occurring in 36.4 days.

Comparison of total gas production between the aerobically treated and untreated HMX hydrolysate treatments at 90 days using a two sample t-test assuming equal variances yielded a test statistic of 1.74. This was less than the

table value of 2.13. The null hypothesis could not be rejected and it could not be concluded that the treatments had different means.

Total gas evolved from the two HMX hydrolysate data sets was compared to the combined data set from the control reactors. Comparison of the treated hydrolysate yielded a test statistic of 2.01 which was less than the table value of 2.37 for a one tailed test. Comparison of the untreated hydrolysate yielded a test statistic of 4.61 which was greater than the table value of 2.37 and led to the rejection of the null hypothesis. It was concluded that a higher level of total gas production was evident in the systems receiving untreated HMX hydrolysate compared to the control treatment.

Comparisons of the time required to achieve 90% of total gas evolved at 90 days between the two hydrolysates and the control data set were made. Comparison of the treated and untreated HMX hydrolysate yielded a test statistic of 15.5. This exceeded the table value of 2.13. The null hypothesis of equal means was rejected and it was concluded that a greater volume of gas was evolved by the systems receiving the untreated HMX hydrolysate.

Comparison of the treated hydrolysate with the combined control data set for the time to produce 90% of the gas evolved yielded a test statistic of 1.06. This was less than the table value of 1.90 ($\alpha = 0.05$, $df = 7$). The null hypothesis could not be rejected and it was concluded that the means were not different. Comparison of the untreated HMX hydrolysate yielded a test statistic of 25.9 which exceeded the table value of 1.90 ($\alpha = 0.05$, $df = 4$). The null hypothesis was rejected and it was concluded that the two means were different.

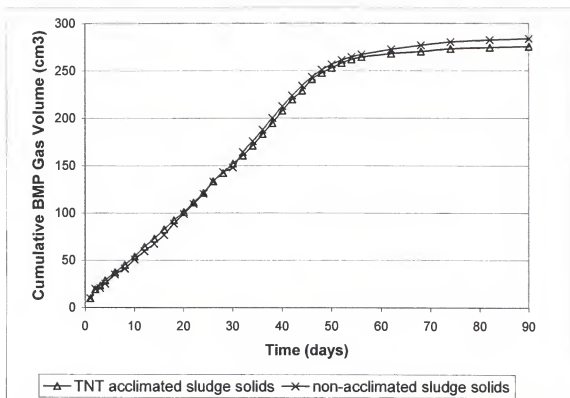


Figure 4-8. Cumulative gas generation versus time for HMX treatment of BMP assays with TNT acclimated or non-acclimated sludge solids.

Analysis of Raw and Denitrified RDX Hydrolysate Treatments

BMP gas generation results from treated and untreated RDX hydrolysate are presented in Figure 4-10 below. Treated RDX hydrolysate produced an average of 277.8 cm^3 of gas in 90 days with 90% of that occurring in 18.0 days. Untreated RDX hydrolysate produced an average of 286.5 cm^3 with 90% of that occurring in 45.5 days.

Comparison of total gas production between the activated sludge treated and untreated HMX hydrolysate at 90 days using a two sample t-test assuming equal variances at a 95% confidence level yielded a test statistic of 3.11. This

exceeded the table value of 1.90 ($\alpha = 0.05$, $df = 7$). The null hypothesis was rejected and it was concluded that the two means were different.

Cumulative gas generation for the activated sludge treated and untreated RDX hydrolysate treatments were compared to the combined data set from the control reactors. Statistical analysis yielded a test statistic of 1.74 which was less than the table value of 1.90 ($\alpha = 0.05$, $df = 7$). The null hypothesis could not be rejected indicating that no difference existed in total gas generation.

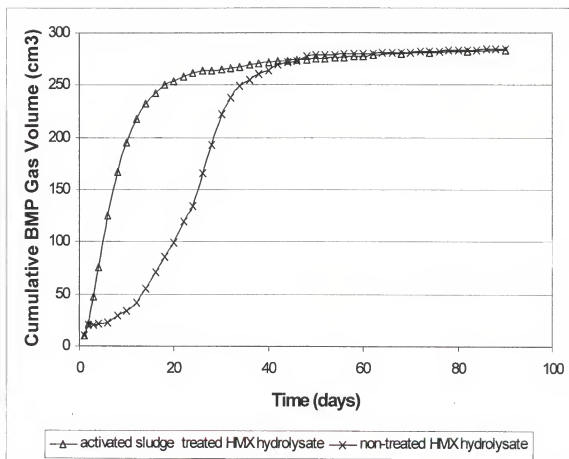


Figure 4-9. Cumulative gas generation versus time for BMP assay's receiving activated sludge treated or raw HMX hydrolysate treatments.

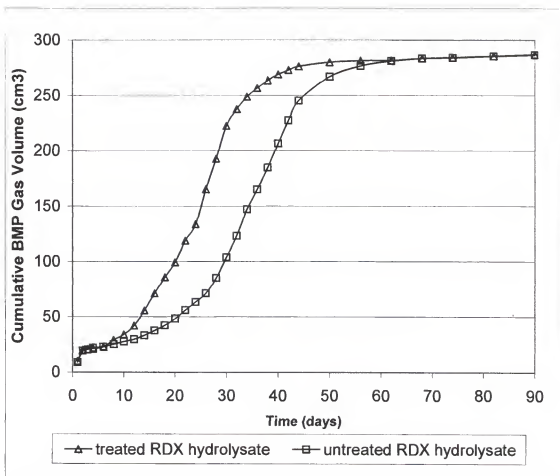


Figure 4-10. Cumulative gas generation versus time for BMP assay's receiving activated sludge treated and raw RDX hydrolysate treatments.

Comparison of the untreated RDX hydrolysate yielded a test statistic of 4.13 which was greater than the table value of 1.90 ($\alpha = 0.05$, $df = 4$) and led to the rejection of the null hypothesis. It was concluded that a higher level of total gas production was evident in the systems receiving untreated HMX hydrolysate compared to the control treatment BMP assays.

Comparison of the time required to achieve 90% of the total gas evolved at 90 days between the aerobically treated and untreated hydrolysates with the control data were made. Statistical analysis yielded a test statistic of 20.6 which

exceeded the table value of 2.13 ($\alpha = 0.05$, $df = 4$). The null hypothesis of equal means was rejected and it was concluded that a difference between the treated RDX hydrolysate and untreated RDX hydrolysate existed.

Comparison of the activated sludge treated hydrolysate with the combined data set from the control treatments yielded a test statistic of 0.41. This was less than the table value of 1.90 and the null hypothesis of equal means could not be rejected. Comparison of the untreated RDX hydrolysate yielded a test statistic of 11.8 which exceeded the table value of 1.90. The null hypothesis was rejected and it was concluded that the untreated RDX hydrolysate treatment resulted in a longer period to achieve 90% of the total gas evolved.

High Anaerobic Solids Assay

High anaerobic solids (HAS) assays were employed to investigate the transformation of TNT, RDX, HMX and a mixture of the three in high solids anaerobic systems. The TNT, RDX, and HMX treatments consisted of 100 ppm of the compound under investigation. The mixed explosive treatment consisted of 33.3 ppm of each of the explosives.

Analysis of Anaerobic TNT Treatment

TNT and byproduct concentrations as a function of time for the non-acclimated and TNT acclimated sludges are presented in Figure 4-11 and Figure 4-12, respectively, below. A graphical comparison of TNT concentration and transformation rates for the TNT acclimated and non-acclimated sludges is presented in Figures 4-13 and 4-14. Raw data values are presented in

Appendix E and Minitab statistical analyses are presented in Appendix I. A summary of statistical data is presented in Table 4-7.

Statistical analysis of log transformed TNT data from control and experimental systems using the mathematical model developed in Chapter 3 yielded an F statistic of 15.9. This exceeded the table value of 5.98 ($\alpha = 0.05$; $df = 1, 18$) for a one-tailed analysis. The null hypothesis of equal slopes was rejected and it was concluded that the systems receiving the TNT acclimated sludge solids transformed TNT at a greater rate compared to systems receiving the non-acclimated sludge solids.

Table 4-7. Statistical analyses of high anaerobic solids assay data.

Parameter	Comparison	SSE ₁	df ₁	MSE ₁	SSE ₂	SSE ₂ - SSE ₁	F _{calc.}	F _{table}
TNT	sludge type	371	18	20.6	699	328	15.88	4.35
Unknown	sludge type	807	20	40.3	830	23.3	0.57	4.35
RDX	sludge type	299	15	20.0	330	30.3	1.51	4.38
HMX	sludge type	882	16	55.1	938	57.1	1.03	4.49
TNT	between mixed and TNT treatments for non-acclimated sludge solids	184	14	13.1	348	164.3	12.53	4.6
RDX	Mixed treatment versus RDX treatment: combined sludge solids data sets	944	24	39.3	1090	146	3.71	4.26
HMX	mixed explosive and TNT treatments	1430	11	130.4	1430	0	0	4.84

A graphical comparison of transformation rates for the unidentified byproduct with TNT acclimated and non-unacclimated sludge solids is presented in Figure 4-15. Raw data values are presented in Appendix D and Minitab statistical analyses are presented in Appendix I. Statistical analysis of log transformed data using the mathematical model developed in Chapter 3 yielded an F statistic of 0.58. This was less than the table value of 5.35 ($\alpha = 0.05$; $df = 1, 20$). The null hypothesis of equal slopes could not be rejected and it was concluded that no differences in transformation rates were evident..

A graphical comparison of ADNT transformation rates for TNT acclimated and non-acclimated sludge solids is presented in Figure 4-16. Raw data values are presented in Appendix D. The non-linear nature of ADNT transformation in the non-acclimated systems did not permit statistical analysis. However, it was observed that complete ADNT transformation required approximately 4.5 times longer in the non-acclimated systems versus the TNT acclimated systems.

Analysis of Anaerobic RDX Treatment

RDX concentrations as a function of time for the non-acclimated and TNT acclimated sludge solids are presented in Figure 4-17 and Figure 4-18. Raw data values are presented in Appendix E. Minitab statistical analyses are presented in Appendix I and summarized in Table 4-7 above.

Transformation of RDX in the TNT acclimated and non-acclimated sludge solids appeared linear in the 20 to 100 ppm range. Plots of concentration as a function of time were regressed and the results are presented below in Table 4-8. The TNT-acclimated and non-acclimated plots had slopes of -0.0783 mg/L/min

and -0.0932 mg/L/min and intercepts of 99.4 mg/L and 84.9 mg/L, respectively. RDX treatment rates for the TNT-acclimated and non-acclimated sludge solids were calculated to be 0.11 g/l/day and 0.13 g/l/day.

Statistical analysis of the slopes of the two data sets using the mathematical model presented in Chapter 3 yielded an F statistic of 1.51. This was less than the table value of 4.38 ($\alpha = 0.05$; $df = 1, 15$). The null hypothesis could not be rejected indicating no differences in the slopes of the two data sets.

Analysis of Anaerobic HMX Treatment

HMX concentrations as a function of time for the non-acclimated and TNT acclimated sludge solids are presented in Figure 4-19 and Figure 4-20. Raw data values are presented in Appendix E. Minitab statistical analyses are presented in Appendix D and summarized in Table 4-7 above.

Transformation of HMX in the TNT acclimated and non-acclimated sludge solids assays was linear in the 10 to 100 ppm range as observed in the RDX assays. Plots of concentration as a function of time were regressed and the results are presented in Table 4-5. Both the TNT acclimated and non-acclimated plots had slopes of -0.0176 mg/L/min and intercepts of 72.6 mg/L and 74.6 mg/L, respectively. The rates of HMX treatment for the TNT-acclimated and non-acclimated sludge solids were determined to be 0.026 g/l/day.

Statistical analysis of the slopes of the two data sets using the mathematical model presented in Chapter 3 yielded an F statistic of 1.03. This was less than the table value of 4.49 ($\alpha = 0.05$; $df = 1, 16$). The null hypothesis could not be rejected indicating no differences in the regression slopes.

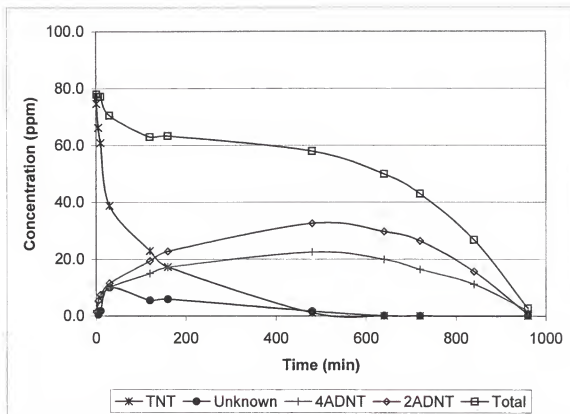


Figure 4-11. Concentration of TNT and byproducts versus time for TNT treatments of high anaerobic solids assays with non-acclimated sludge solids.

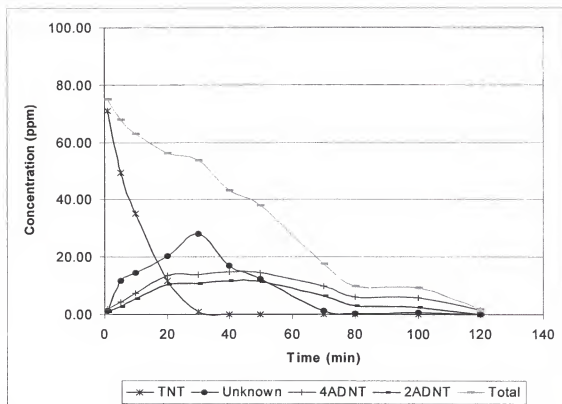


Figure 4-12. Concentration of TNT and byproducts versus time for TNT treatments of high anaerobic solids assays with TNT acclimated sludge solids.

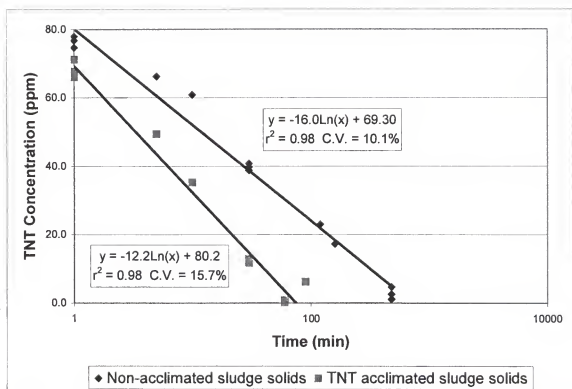


Figure 4-13. Concentration of TNT versus time for TNT treatments of high anaerobic solids assays with TNT acclimated or non-acclimated sludge solids.

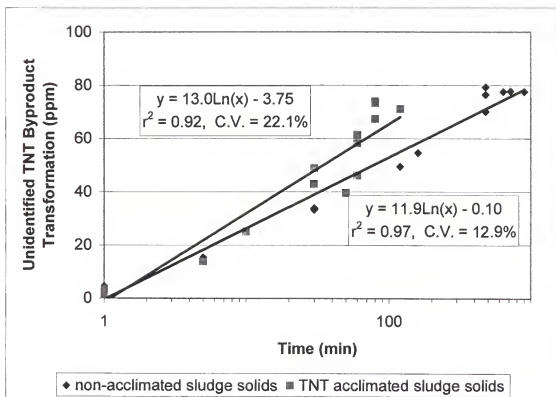


Figure 4-14. Unidentified TNT byproduct transformation versus time for TNT treatments of high anaerobic solids assays with TNT acclimated and non-acclimated sludge solids.

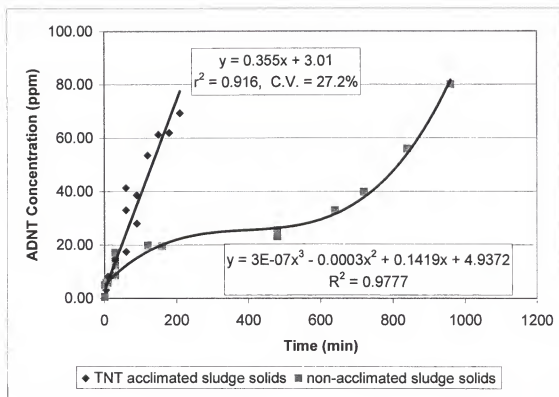


Figure 4-15. Aminodinitrotoluene transformation versus time for TNT treatments of high anaerobic solids assays with TNT acclimated or non-acclimated sludge solids.

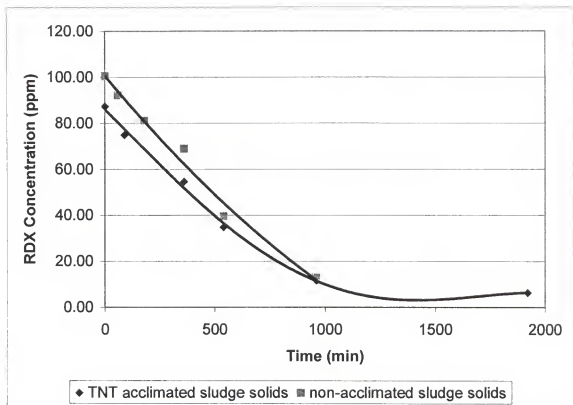


Figure 4-16. Concentration of RDX versus time for RDX treatment of high anaerobic solids assays with TNT acclimated or non-acclimated sludge solids.

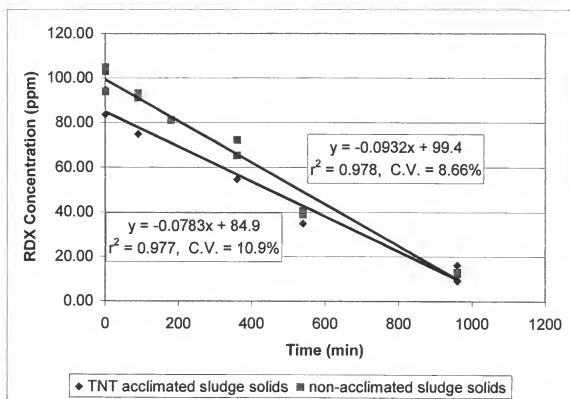


Figure 4-17. Regression curves and equations for the linear portions of the RDX data sets.

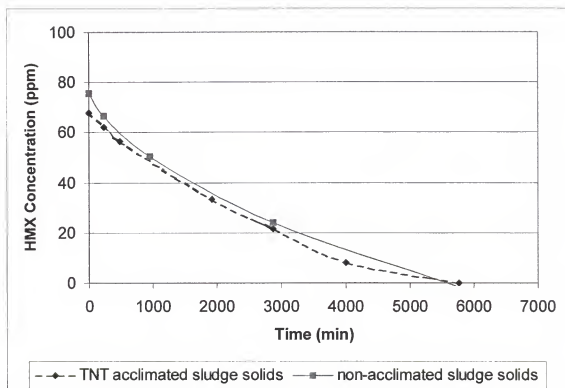


Figure 4-18. Concentration of HMX versus time for HMX treatments of high anaerobic solids assays with TNT acclimated or non-acclimated sludge solids.

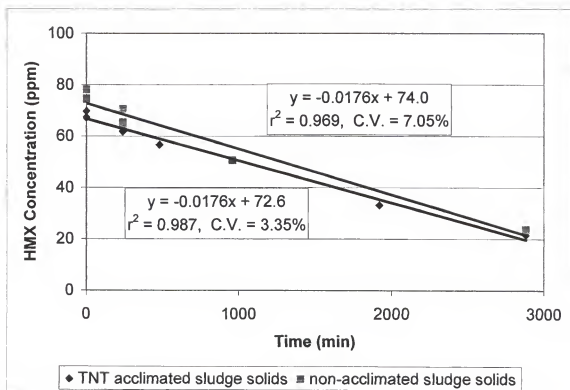


Figure 4-19. Regressed curves and equations for the linear portions of the HMX concentration plots.

Analysis of Anaerobic TNT, RDX, and HMX Mixture Treatment

Concentrations of RDX, HMX, TNT and TNT byproducts are presented in Figures 4-21 to 4-26 below for assays receiving a mixed treatment containing all three compounds. Data points for systems receiving only one compound are presented for comparison. No statistically significant differences in transformation rates were observed between the data sets when TNT acclimated sludge solids and non-acclimated sludge solids were compared.

Comparison of the rate of TNT transformation in systems receiving multiple compounds versus only TNT in non-acclimated sludge solids systems yielded an F statistic of 12.5. This exceeded the Table value of 4.6 ($\alpha = 0.05$, $df = 1, 14$); therefore, the null hypothesis of equal slopes was rejected. It was concluded that the steeper slope of the transformation curve in non-acclimated reactors was significantly different than in systems receiving multiple compounds.

Comparison of the rate of RDX transformation in systems receiving multiple compounds versus only RDX for combined sludge data sets yielded an F statistic of 3.71. This did not exceed the table value of 4.26 ($\alpha = 0.05$, $df = 1, 24$). It was concluded that no RDX transformation rate differences were evident between the RDX and mixed explosive treatments.

Comparison of the rate of HMX transformation in systems receiving the mixed explosive treatment containing 33.3 ppm HMX with the HMX treatment generated an F statistic of 0.002 which was less than the table value of 4.84. The null hypothesis could not be rejected indicating that no significant difference was evident in the data sets.

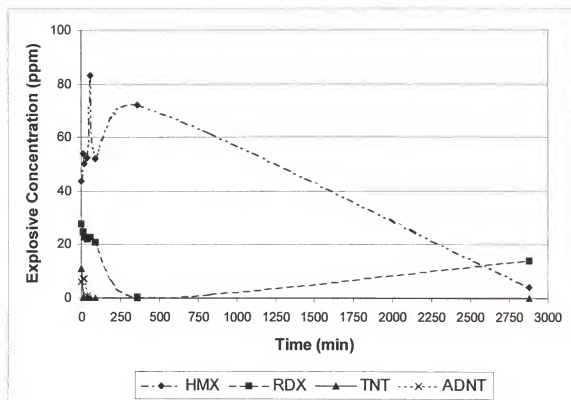


Figure 4-20. Concentrations of TNT, RDX, HMX and ADNT versus time for mixed explosive treatment of high anaerobic solids reactors with TNT acclimated sludge solids.

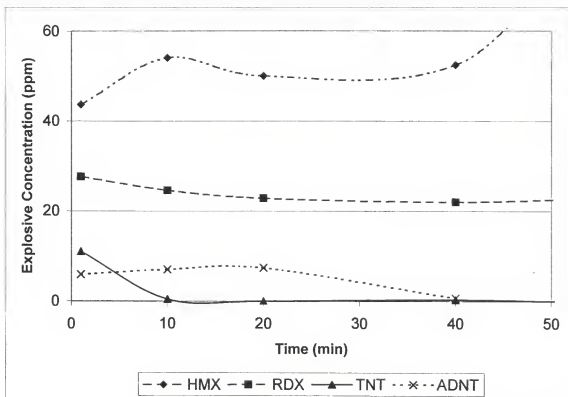


Figure 4-21. Concentrations of TNT, RDX, HMX and ADNT versus time for mixed explosive treatments of high anaerobic solids reactors with TNT acclimated sludge solids.

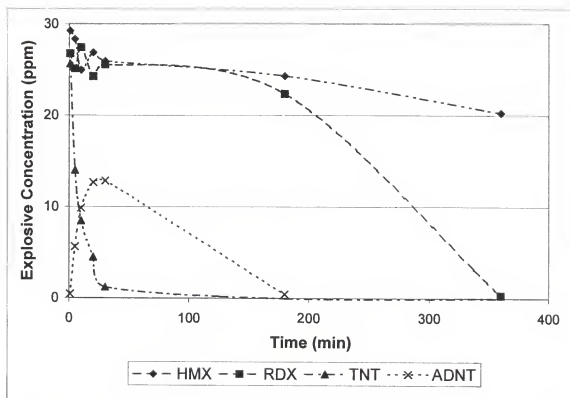


Figure 4-22. Concentrations of TNT, RDX, HMX and ADNT versus time for mixed explosive treatments of high anaerobic solids reactors with non-acclimated sludge solids.

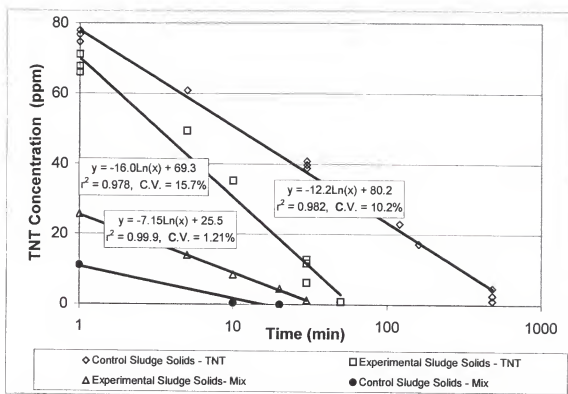


Figure 4-23. Concentration of TNT versus time for TNT and mixed explosive treatments of high anaerobic solids assays with TNT acclimated or non-acclimated sludge solids showing regression curves and equations.

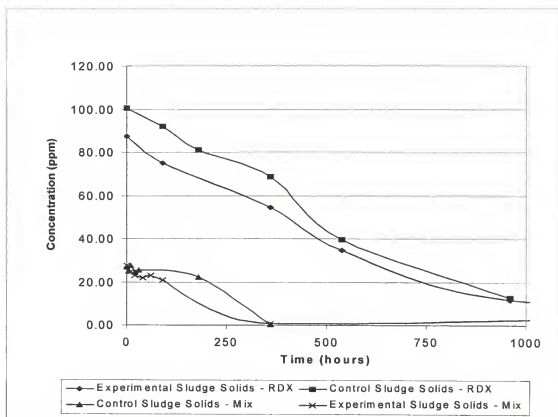


Figure 4-24. Concentration of RDX versus time for RDX treatments of high anaerobic solids assays with TNT acclimated (experimental) or non-acclimated (control) sludge solids.

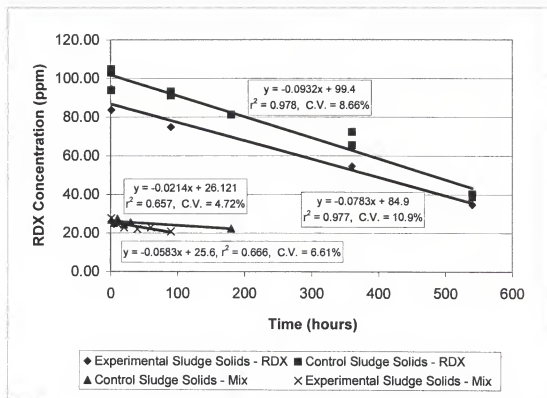


Figure 4-25. Concentration of RDX versus time for RDX and mixed explosive treatments of high anaerobic solids assays with TNT acclimated (experimental) or non-acclimated (control) sludge solids showing regression curves and equations.

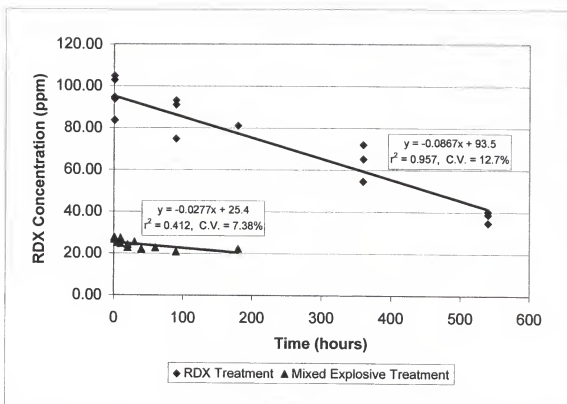


Figure 4-26. Concentration versus time for RDX and mixed explosive treatments of high anaerobic solids assays with combined sludge data sets showing regression curves and equations.

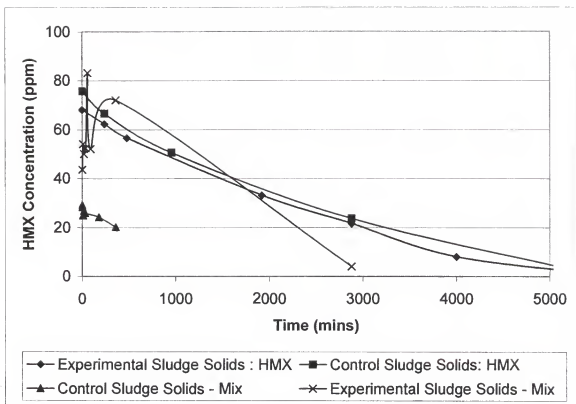


Figure 4-27. Concentration of HMX versus time for HMX and mixed explosive treatments of high anaerobic solids assays with TNT acclimated (experimental) or non-acclimated (control) sludge solids.

CHAPTER 5 SUMMARY AND CONCLUSION

Transformation of the high energy compounds 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) were investigated in high anaerobic solids systems. Additionally, treatment of the products of alkaline hydrolysis of RDX and HMX were investigated in activated sludge systems. The effects of TNT, RDX, HMX, and the raw and activated sludge treated hydrolysates on gas generation rates in biochemical methane potential assays were determined.

One liter activated sludge reactors repeatedly denitrified RDX and HMX hydrolysates over a 24 day period, accomplishing complete (>99%) nitrite removal with greater than 65% COD reduction. Nitrite removal was linear up to 500 mg/L/day dose rate for at least 24 days.

Average total solids concentration in the RDX hydrolysate activated sludge reactor were reduced from 7200 mg/L to 5600 mg/L over the experimental period. The sludge had an initial volatile solids fraction of 0.78. The reactor removed 95.0% of the nitrite (500 ppm dose level) from a 22.8 mL dose of RDX hydrolysate in 8 hours. Data regression indicated nitrite was removed from the system at a rate of 55.4 mg/L/hr in the linear portion of the curve.

Average total solids concentration in the HMX hydrolysate activated sludge reactor were reduced from 7500 mg/L to 6050 mg/L over the experimental

period. The sludge had an initial volatile solids fraction of 0.76. The reactor removed 93.9% of the nitrite (500 ppm dose level) from a 33.2 mL dose of HMX hydrolysate in 5 hours. Data regression indicated the removal of nitrite occurred at a rate of 88.3 mg/L/hr in the linear portion of the curve.

The treatment rate for RDX using hydrolysis followed by activated sludge was calculated to be 95 mg/L/hr in the combined treatment process. The treatment rate for HMX using hydrolysis followed by activated sludge was calculated to be 152 mg/L/hr HMX in the combined treatment process.

The HMX hydrolysate solution contained 0.04 mg/L of unhydrolyzed HMX. No HMX was detected in the activated sludge reactor receiving HMX hydrolysate at the end of the 24 day treatment period indicating that the system was capable of denitrifying in the presence of residual HMX, while transforming small amounts of the parent compound. No RDX was detected in the RDX hydrolysate pre- or post- treatment.

Comparison of total gas generation from BMP assays between TNT acclimated and non-acclimated sludge solids did not indicate any differences in total gas production (V_{total}) for control, TNT, RDX, and HMX treatments. Analysis of the time required to generate 90% of the total gas produced ($t_{90\%}$) for the four treatments indicated a slight difference between sludge types in systems which received the RDX treatment ($P = 0.026$). The $t_{90\%}$ was 75.4 days for TNT acclimated systems and 70.6 days for non-acclimated systems indicating that sludge acclimation resulted in lower gas evolution rates in RDX treatments.

A difference in total gas production (V_{total}) was noted in the comparisons of TNT treatments to Control treatments indicating a significant inhibitory effect on total gas production in the presence of TNT. The $t_{90\%}$ for RDX and HMX treatments of 73.0 and 47.7 days respectively were found to differ significantly from the control treatment $t_{90\%}$ of 18.6 days indicating a significant delay in the onset of gas production from these treatments.

Untreated RDX and HMX hydrolysate additions produced a greater volume of total gas compared to controls and delayed gas evolution ($t_{90\%} = 45.5$ and 36.4 days, respectively). Activated sludge treatment of RDX and HMX hydrolysates prior to BMP assay investigations eliminated differences in gas evolution rates between the control and hydrolysate treatments. This result indicated the potential for reducing the inhibitory effects of RDX and HMX hydrolysates on anaerobic microorganisms through activated sludge pretreatment for nitrite removal.

The compounds TNT, RDX, and HMX were found to be amenable to transformation under anaerobic conditions in 100 mL high anaerobic solids (HAS) assays. Sludge acclimation to TNT increased TNT transformation rates by 37% compared to non-acclimated systems. The TNT acclimated systems required 30 minutes to transform 59 ppm TNT versus 43 ppm in non-acclimated systems. Additionally, TNT acclimated systems removed aminodinitrotoluene (ADNT) transformation byproducts in 120 minutes versus 960 minutes for the non-acclimated systems. Comparison of TNT transformation in non-acclimated sludge solids systems receiving TNT treatments versus multiple explosive

compounds treatments indicated an inhibitory effect of RDX and HMX on TNT transformation .

No difference in transformation rates between the TNT acclimated and non-acclimated sludge types were detected for the RDX and HMX treatments in the high anaerobic solids systems indicating that sludge acclimation to TNT was not effective in enhancing the degradation rates of these compounds. Removal of an initial 100 ppm RDX dose occurred in 960 minutes for both sludge types. Additionally, complete removal of an initial 100 ppm dose of HMX dose occurred within 5760 minutes for both sludges. Regression of RDX and HMX curves for the high anaerobic solids systems yielded treatment potentials of 5.1 mg/L/hr and 1.1mg/L/hr, respectively. The transformation rates for RDX and HMX were found to be significantly greater than those presented in the literature indicating the potential for using high anaerobic solids systems in the treatment of these compounds.

The treatment of RDX using hydrolysis followed by activated sludge (AS) compared to high anaerobic solids treatment indicated 18 times the treatment rate in the AS system. The treatment of HMX using hydrolysis followed by activated sludge (AS) compared to high anaerobic solids treatment indicated 140 times the treatment within the AS system.

Proposed Future Work

Anaerobic treatment of the effluent from the activated sludge reactors should be further evaluated using the modified BMP protocol. Gas production, COD, and the concentration of RDX and HMX should be monitored to assess

inhibitory effects of the anoxically/aerobically treated hydrolysates, to determine the ultimate degradability of remaining COD and to demonstrate that no accumulation of RDX or HMX occurs within the systems.

Optimization of operating parameters should be performed in scaled up reactors and include waste feed rates and electron donor feed rates. Additionally, acclimation of anaerobic sludge to both RDX and HMX should be investigated to enhance degradation rates.

APPENDIX A
SAMPLE CALCULATIONS

Activated Sludge Reactor Design

Hydrolysate Properties

	HMX	RDX
COD mg/L	19200	35900
BOD mg/L	9100	10100
Nitrite	16060	23347
Nitrate	1092	1086
Chloride	7478	4856

HMX Hydrolysate

Target Dilution	4000 g/m3	Cl-	(Based on HMX hydrolysate)
Dilution Factor	7478 = 4000	1.8695 =	2

i.e. Add 1 mL water (mineral) per mL hydrolysate

Dosing

16060 g/m3 NO2	*	14/46	=	4888 g/m3 NO2-N
			=	0.004888 g/mL NO2-N

Peak Denitrification Rate

From Graph provided by Dr. Koopman

0.09 g NO3-N reduced g MLVSS * day	0.75 g VSS g TSS	3000 g TSS m3
=	162 g NO3-N reduced m3 * day	= 0.162 g NO3-N Liter * day

Daily Dose

0.162 g/d NO2- N	=	33.14357 mL/day
0.004888 g/mL NO2-N		
33.14357 mL hydrolysate		
33.14357 mL water		

$$\begin{aligned} (1/5)(\text{NO}_3^-) + (6/5)(\text{H}^+) + (\text{e}^-) &= (1/10)(\text{N}_2) + (3/5)(\text{H}_2\text{O}) \\ (1/4)(\text{NO}_2^-) + (\text{H}^+) + (\text{e}^-) &= (1/8)(\text{N}_2) + (1/2)(\text{H}_2\text{O}) \end{aligned}$$

$$\begin{aligned} &4 \text{ g COD} && (1/5)(\text{NO}_3^-) && \text{e}^- \\ &\text{g NO}_3\text{-N red. to N}_2 && \text{e}^- && (1/4)(\text{NO}_2^-) \\ \\ = &3.2 && \text{g COD} && \\ &&& \text{g NO}_2\text{-N red. to N}_2 && \\ \\ 4*1092 + 3.2*16060 &= && 27880 \text{ COD Needed} && \\ &2 && && \end{aligned}$$

9600 COD Present

18280 COD Differential

33.2 mL hydrolysate

33.2 mL @

18280 mg/L COD

RDX Hydrolysate

Dilution Factor

2

Calculated above for HMX

i.e. Add 1 mL water (mineral) per mL hydrolysate

Dosing

23350 g/m3
NO2

*

14/46

=

7106 g/m3 NO2-N

=

0.007106 g/mL NO2-N

Peak Denitrification Rate (Determined above under HMX Section)

0.162 g NO3-N reduced

Liter * day

Daily
Dose

0.162 g/d NO2- =
N

22.8 mL/day

0.00712 g/mL NO2-N

22.8 mL hydrolysate

22.8 mL water

$(1/5)(\text{NO}_3^-) + (6/5)(\text{H}^+) + (\text{e}^-) = (1/10)(\text{N}_2) + (3/5)(\text{H}_2\text{O})$

$(1/4)(\text{NO}_2^-) + (\text{H}^+) + (\text{e}^-) = (1/8)(\text{N}_2) + (1/2)(\text{H}_2\text{O})$

4 g COD

g NO3-N red. to N2

$(1/5)(\text{NO}_3^-)$

e-

$(1/4)(\text{NO}_2^-)$

=

3.2

g COD

g NO2-N red. to N2

$4 \times 1086 + \frac{3.2 \times 23350}{2} =$

39530 COD Needed

17950 COD Present

21580 COD Differential

22.8 mL hydrolysate

22.8 mL @

21580 mg/L COD

APPENDIX B
ANAEROBIC DIGESTER AND ACTIVATED
SLUDGE DIGESTER DATA

Table B-1. TNT acclimated sludge solids data for anaerobic reactor 1.

Date	pH	Eh (mV)	Cond (mho)	Temp (C)	Food (g)	Gas (mL)	DI (mL)	Other
10/21/1999	7.5	-315	10.3	35.5				
10/22/1999	7.55	-315	10.1	35.4				
10/23/1999	7.55	-310	10.3	35.5	6		100	-500mL sludge
10/24/1999	7.1	-320	9.3	35.6				
10/25/1999	7.2	-300	9.4	35.8				
10/26/1999	7.3	-305	9.2	35.5				
10/27/1999	7.45	-310	9.5	35.4				
10/28/1999	7.45	-310	9.5	35.5				
10/29/1999	7.4	-300	9.4	35.3				
10/30/1999	7.4	-305	9.6	34.7	6		100	
10/31/1999	7	-295	8.8	34.9				
11/1/1999	7.15	-300	8.9	35.5				
11/2/1999	7.2	-310	9	36.3				
11/3/1999	7.4	-305	8.8	35.9				
11/4/1999	7.5	-310	9	35.7				
11/5/1999	7.45	-305	9.1	35.5				
11/6/1999	7.45	-305	9.3	35.3	6	1100	50	
11/7/1999	7	-300	9.2	34.6		1350		
11/8/1999								
11/9/1999	7.35	-295	9.5	34.5		1480		
11/10/1999	7.4	-300	9.5	35		260		
11/11/1999	7.45	-300	9.4	34.7		200		0.0509 g TNT
11/12/1999	7.45	-310	9.5	34.6		220		in 4mL MeOH
11/13/1999	7.45	-310	9.6	34.9	6	1340	50	
11/14/1999	7	-300	9.7	35.3		1060		
11/15/1999	7.1	-295	9.7	35		1100		
11/16/1999	7.2	-300	9.5	34.8		440		
11/17/1999	7.3	-305	9.6	34.8		220		
11/18/1999	7.35	-305	9.8	34.7		160		
11/19/1999	7.4	-310	10	34.6		140		
11/20/1999	7.45	-315	10.1	35.5	6	1020	50	
11/21/1999	7	-315	9.9	35.1		1250		
11/22/1999	7.05	-300	10	34.8		960		
11/23/1999	7.1	-310	10.3	34.9		600		
11/24/1999	7.2	-315	10.4	35		340		
11/25/1999	7.3	-305	10.2	46.6		200		0.0512 g TNT
11/26/1999						220		in 4mL MeOH
11/27/1999	7.35	-300	10.3	35.2	6	1500	50	
11/28/1999	6.9	-310	10.5	35.3		1050		
11/29/1999	7	-320	10.6	35.1		750		
11/30/1999	7.15	-320	10.5	35		600		
12/1/1999								
12/2/1999								
12/3/1999	7.25	-320	10.4	35.3		750		
12/4/1999	7.3	-315	10.6	35.5	6	1180	50	
12/5/1999	7	-305	10.6	34.9		1040		
12/6/1999	7.1	-300	10.8	34.7		1200		
12/7/1999	7.25	-305	10.6	35.2		700		

Table B-1. TNT acclimated sludge solids data for anaerobic Reactor 1 (continued).

Date	pH	Eh (mV)	Cond (mho)	Temp (C)	Food (g)	Gas (mL)	DI (mL)	Other
12/8/1999	7.35	-310	10.9	35		240		
12/9/1999	7.4	-305	11	35.2		300		0.0506 g TNT in 4mL MeOH
12/10/1999						140		
12/11/1999	7.45	-310	11.2	35.3	6	1180	50	
12/12/1999	7.1	-315	11.1	35		1240		
12/13/1999	7.2	-310	11.3	35.1		1000		
12/14/1999								
12/15/1999	7.35	-305	11.5	34.9		1060		
12/16/1999	7.45	-310	11.6	34.7		180		
12/17/1999	7.25	-315	11.4	34.8		160		
12/18/1999	7.4	-320	11.5	35.2	6	920	100	-500 mL sludge
12/19/1999	7.15	-305	10.8	35		1280		
12/20/1999	7.35	-310	10.6	34.6		1080		
12/21/1999	7.15	-310	10.9	34.9		380		
12/22/1999								
12/23/1999								
12/24/1999						860		
12/25/1999	7.45	-315		35.3	6	1000	100	
12/26/1999								
12/27/1999	7.15	-315		34.9		1980		
12/28/1999	7.3	-320		35.3		940		
12/29/1999								
12/30/1999								
12/31/1999	7.5	-315		34.9		580		
1/1/2000	7.5	-320		35	6	880	50	
1/2/2000	7.05	-310		35.1		1200		
1/3/2000	7.15	-315		35		1120		
1/4/2000	7.3	-315		35.5		580		
1/5/2000	7.35	-320		34.8		340		
1/6/2000	7.45	-320		34.6		260		
1/7/2000	7.5	-320		34.9		140		
1/8/2000	7.55	-315		35.2	6	960	50	
1/9/2000	7.1	-300		35.1		1250		
1/10/2000	7.2	-315		35.4		1000		
1/11/2000	7.25	-320		35.6		720		
1/12/2000	7.35	-320		35.3		440		
1/13/2000	7.4	-320		34.9		200		
1/14/2000						160		
1/15/2000	7.55	-310		34.9	6	1020	50	
1/16/2000	7	-310		34.7		1320		
1/17/2000	7.15	-300		34.6		980		
1/18/2000	7.35	-305		34.7		860		
1/19/2000								
1/20/2000								
1/21/2000						540		
1/22/2000	7.5	-320		34.6	6	1300	50	
1/23/2000	7.1	-300		34.8		1240		
1/24/2000	7.2	-310		34.7		1020		

Table B-1. TNT acclimated sludge solids data for anaerobic reactor 1 (continued).

Date	pH	Eh (mV)	Cond (mho)	Temp (C)	Food (g)	Gas (mL)	DI (mL)	Other
1/25/2000								
1/26/2000								
1/27/2000								
1/28/2000						1080		
1/29/2000	7.45	-320		35.1	6	1280	50	
1/30/2000	6.95	-315		35.1		1180		
1/31/2000	7.1	-310		35.2		1040		
2/1/2000	7.3	-315		35.1		480		
2/2/2000	7.4	-320		35.1		240		
2/3/2000	7.5	-315		35.3		220		
2/4/2000	7.55	-305		34.1		180		
2/5/2000	7.55	-310		35.4	6	1320	50	
2/6/2000	7.1	-320		34.5		1280		
2/7/2000	7.2	-315		35.6		1020		
2/8/2000	7.35	-305		35.8		560		
2/9/2000	7.45	-300		36.5		320		
2/10/2000	7.5	-315		35.8		160		
2/11/2000	7.45	-310		35.7		180		
2/12/2000	7.5	-320		35.2	6	780	100	-500 mL sludge
2/13/2000	7.05	-310		35.5		1080		
2/14/2000	7.15	-310		35.6		1260		
2/15/2000								
2/16/2000								
2/17/2000								
2/18/2000						1300		
2/19/2000	7.45	-305		35.4	6	940	100	
2/20/2000	6.95	-320		35.1		1120		
2/21/2000	7.05	-310		35.5		1380		
2/22/2000	7.2	-310		35.9		600		
2/23/2000								
2/24/2000								
2/25/2000						540		
2/26/2000	7.5	-295		35.4	6	1020	50	
2/27/2000	7	-315		35.5		1240		
2/28/2000	7.1	-305		34.3		1400		
2/29/2000	7.15	-310		34.8		640		
3/1/2000								
3/2/2000								
3/3/2000						380		
3/4/2000	7.5	-310		35	6	1120	50	
3/5/2000	7.1	-310		35.3		1420		
3/6/2000	7.25	-320		35.4		1320		
3/7/2000								
3/8/2000								
3/9/2000								
3/10/2000						740		
3/11/2000	7.55	-310		35.1	6	1160	50	
3/12/2000	7.1	-315		34.6		1380		

Table B-1. TNT acclimated sludge solids data for anaerobic reactor 1 (continued).

Date	pH	Eh (mV)	Cond (mho)	Temp (C)	Food (g)	Gas (mL)	DI (mL)	Other
3/13/2000								
3/14/2000								
3/15/2000								
3/16/2000								
3/17/2000	7.5	-315		34.7		2100		
3/18/2000	7.5	-315		35.1	6	1260	50	
3/19/2000	7.05	-310		34.2		1440		
3/20/2000	7.25	-305		34.6		1000		
3/21/2000								
3/22/2000								
3/23/2000								
3/24/2000	7.45	-305		34.1		880		
3/25/2000	7.55	-310		34.8	6	1340	50	
3/26/2000	7.15	-320		35		1480		
3/27/2000	7.25	-315		34.9		1020		
3/28/2000								
3/29/2000								
3/30/2000								
3/31/2000						720		
4/1/2000	7.45	-310		34.8	6	1320	50	
4/2/2000	7	-315		34.6		1340		
4/3/2000	7.25	-315		34.4		1160		
4/4/2000								
4/5/2000								
4/6/2000	7.55	-310		35.8		680		
4/7/2000	7.55	-315		34		160		
4/8/2000	7.6	-310		34.9	6	880	100	500 mL sludge removed.
4/9/2000	7.1	-315		35		1140		
4/10/2000	7.25	-320		34.5		1260		$V_{\text{reactor}} = 1.0 \text{ L}$
4/11/2000								
4/12/2000								
4/13/2000								
4/14/2000						1320		
4/15/2000	7.4	-320		33.6	6	960	100	
4/16/2000	6.75	-315		34.5		1080		
4/17/2000	6.95	-320		34.7		1120		
4/18/2000	6.9	-320		34.8		640		
4/19/2000	6.95	-310		35.2		380		
4/20/2000								
4/21/2000	7.1	-320		35.6		440		
4/22/2000	7.5	-315		35.5	6	980	50	
4/23/2000	7.05	-320		35.2		1120		
4/24/2000	7.2	-315		35.7		1160		
4/25/2000	7.25	-320		34.6		520		
4/26/2000	7.35	-320		34.8		300		
4/27/2000	7.4	-315		35.3		180		
4/28/2000	7.5	-315		35.5		180		

Table B-1. TNT acclimated sludge solids data for anaerobic reactor 1 (continued).

Date	pH	Eh (mV)	Cond (mho)	Temp (C)	Food (g)	Gas (mL)	DI (mL)	Other
4/29/2000	7.5	-320		35.4	6	940	50	
4/30/2000	7.1	-320		35.8		1160		
5/1/2000	7.2	-310		36.3		1220		
5/2/2000	7.3	-320		36.5		660		
5/3/2000	7.35	-320		35.9		280		
5/4/2000	7.4	-310		35		180		
5/5/2000	7.45	-320		35.3		200		
5/6/2000	7.55	-315		35.4	6	1000	50	
5/7/2000	7.15	-315		35.7		1280		
5/8/2000	7.25	-320		35.1		1060		
5/9/2000	7.3	-320		34.9		680		
5/10/2000	7.4	-325		34.6		240		
5/11/2000	7.45	-320		34.4		160		
5/12/2000	7.5	-320		34.1		180		
5/13/2000	7.55	-325		34.5	6	1120	50	
5/14/2000	6.95	-315		34.8		1320		
5/15/2000	7.15	-320		34.5		1140		
5/16/2000	7.2	-320		34.4		440		
5/17/2000	7.35	-315		34.8		260		
5/18/2000	7.45	-320		34.3		160		
5/19/2000	7.45	-320		34.2		200		
5/20/2000	7.45	-315	12.5	34.6	6	1180	50	
5/21/2000	7.05	-325	12.4	34.3		1240		
5/22/2000	7.15	-320	12.3	34.4		1180		
5/23/2000	7.25	-315	12.3	34.7		560		
5/24/2000	7.35	-310	12.4	35.1		240		
5/25/2000	7.4	-320	12.6	35.5		200		
5/26/2000	7.5	-320	12.5	35.3		180		
5/27/2000	7.55	-315	12.7	34.8	6	1320	50	
5/28/2000	7.1	-320	12.9	34.4		1340		
5/29/2000	7.25	-320	12.8	34.7		1060		
5/30/2000	7.35	-320	12.9	34.5		560		
5/31/2000	7.4	-320	12.8	34.6		220		
6/1/2000	7.5	-315	13.1	35.4		160		
6/2/2000	7.55	-320	13.1	35.6		200		
6/3/2000	7.55	-315	13.3	35.7	6		100	-500 mL sludge

Table B-2. TNT acclimated sludge solids data for anaerobic reactor 5.

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)	Other
10/21/1999	7.55	-320	9.9	34.3			
10/22/1999	7.5	-310	10.1	34.5			
10/23/1999	7.55	-315	10	34.8	6	100	-500mL sludge
10/24/1999	7.05	-310	9.1	34.7			
10/25/1999	7.15	-310	9.2	35.6			
10/26/1999	7.25	-305	9.4	35.6			
10/27/1999	7.4	-315	9.3	35.7			
10/28/1999	7.4	-300	9.4	35.1			
10/29/1999	7.45	-300	9.4	34.2			
10/30/1999	7.45	-305	9.5	35.6	6	100	
10/31/1999	7	-295	8.6	35.7			
11/1/1999	7.15	-300	8.5	35.4			
11/2/1999	7.25	-200	8.8	35.3			
11/3/1999	7.4	-295	8.9	35.2			
11/4/1999	7.45	-300	8.7	34.8			
11/5/1999	7.45	-305	9	34.6			
11/6/1999	7.45	-305	9.1	34.7	6	960	50
11/7/1999	7.3	-300	9.2	34.7		1180	
11/8/1999							
11/9/1999	7.35	-300	9.3	34.6		1420	
11/10/1999	7.45	-305	9.5	35.1		440	
11/11/1999	7.45	-310	9.4	35.2		260	
11/12/1999	7.4	-305	9.8	35		220	
11/13/1999	7.5	-300	9.5	35	6	1120	50
11/14/1999	7.05	-310	9.6	34.6		1260	
11/15/1999	7.15	-300	9.3	34.7		920	
11/16/1999	7.3	-295	9.5	34.8		480	
11/17/1999	7.45	-290	9.6	34.5		240	
11/18/1999	7.45	-295	9.6	34.9		260	
11/19/1999	7.5	-295	9.5	34.8		180	
11/20/1999	7.45	-305	9.9	35	6	980	50
11/21/1999	7.15	-300	10	35.1		1360	
11/22/1999	7.2	-300	9.9	35.2		950	
11/23/1999	7.25	-305	10.2	35.2		660	
11/24/1999	7.3	-300	10	35.4		240	
11/25/1999	7.35	-310	10.2	35.5		200	
11/26/1999						180	
11/27/1999	7.4	-305	10.3	35.8	6	1200	50
11/28/1999	7.1	-295	10.1	35.3		1200	
11/29/1999	7.25	-300	10.2	34.8		850	
11/30/1999	7.3	-310	10.4	35		550	
12/1/1999							
12/2/1999							
12/3/1999	7.35	-310	10.7	34.5		750	
12/4/1999	7.35	-295	10.5	34.2	6	1140	50
12/5/1999	7.05	-305	10.7	34.5		1320	
12/6/1999	7.2	-300	10.9	35		1060	
12/7/1999	7.35	-310	10.6	35.2		680	

Table B-2. TNT acclimated sludge solids data for anaerobic reactor 5 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)	
12/8/1999	7.3	-310	10.8	35.3	260		
12/9/1999	7.4	-315	10.7	35.4	220		
12/10/1999							0.0511 g TNT in 4mL MeOH
12/11/1999	7.5	-315	10.9	35.2	6	540	50
12/12/1999	7.1	-305	10.8	35.1		1220	
12/13/1999	7.25	-310	10.6	35		1160	
12/14/1999							
12/15/1999	7.35	-310	11.2	35.1		700	
12/16/1999	7.4	-305	11	35.2		1650	
12/17/1999	7.5	-310	11.2	34.9		650	
12/18/1999	7.4	-310	11.3	35.1	6	920	100
12/19/1999	6.9	-315	10.5	35.4		1040	-500 mL sludge
12/20/1999	7	-315	10.7	35.7		900	
12/21/1999	7.15	-310	10.1	35.1		660	
12/22/1999							
12/23/1999							
12/24/1999					880		
12/25/1999	7.45	-310		35	6	940	100
12/26/1999							
12/27/1999	7.15	-320		34.4		2140	
12/28/1999	7.3	-315		34.6		740	
12/29/1999							
12/30/1999							
12/31/1999	7.5	-320		34.7		720	
1/1/2000	7.45	-320		34.6	6	950	50
1/2/2000	6.95	-310		35.7		1260	
1/3/2000	7.1	-315		35.4		1100	
1/4/2000	7.25	-310		35.3		580	
1/5/2000	7.3	-320		35.3		260	
1/6/2000	7.45	-315		35.4		140	
1/7/2000	7.5	-320		35.5		200	
1/8/2000	7.55	-315		35.2	6	1080	50
1/9/2000	7.05	-300		35.5		1120	
1/10/2000	7.15	-315		34.7		1160	
1/11/2000	7.25	-320		34.8		480	
1/12/2000	7.3	-320		35.1		340	
1/13/2000	7.4	-315		34.9		180	
1/14/2000						160	
1/15/2000	7.5	-320		35.6	6	1000	50
1/16/2000	7	-315		35.2		1280	
1/17/2000	7.15	-310		34.8		1000	
1/18/2000	7.35	-320		34.9		640	
1/19/2000							
1/20/2000							
1/21/2000					780		
1/22/2000	7.5	-320		36.3	6	1060	50
1/23/2000	7.1	-315		35.6		1220	
1/24/2000	7.2	-310		35.1		1160	

Table B-2. TNT acclimated sludge solids data for anaerobic reactor 5 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)	Other
1/25/2000							
1/26/2000							
1/27/2000							
1/28/2000					1220		
1/29/2000	7.5	-320		35.9	6	1320	50
1/30/2000	7.05	-315		35.5		1140	
1/31/2000	7.2	-310		35.6		1000	
2/1/2000	7.3	-305		36.3		540	
2/2/2000	7.45	-315		35.6		400	
2/3/2000	7.55	-320		36.2		220	
2/4/2000	7.55	-310		35.1		240	
2/5/2000	7.55	-310		34.4	6	1060	50
2/6/2000	7.1	-315		35.1		1320	
2/7/2000	7.2	-310		35.9		1140	
2/8/2000	7.3	-305		35.4		680	
2/9/2000	7.4	-305		34.8		340	
2/10/2000	7.45	-295		33.7		160	
2/11/2000	7.45	-300		34.6		200	
2/12/2000	7.5	-310		34.3	6	840	100 -500 mL sludge
2/13/2000	7.1	-320		33.7		1100	
2/14/2000	7.2	-305		34.5		1340	
2/15/2000							
2/16/2000							
2/17/2000							
2/18/2000					1060		
2/19/2000	7.4	-315		34.7	6	880	100
2/20/2000	7	-315		34.3		1240	
2/21/2000	7.1	-310		35.6		1280	
2/22/2000	7.2	-315		34.2		540	
2/23/2000							
2/24/2000							
2/25/2000					480		
2/26/2000	7.45	-315		34.9	6	960	50
2/27/2000	7.05	-310		34.3		1180	
2/28/2000	7.15	-310		34.6		1320	
2/29/2000	7.3	-305		35.4		640	
3/1/2000							
3/2/2000							
3/3/2000					460		
3/4/2000	7.45	-315		34.7	6	1120	50
3/5/2000	7.5	-315		34.6		1280	
3/6/2000	7.45	-310		35.8		1240	
3/7/2000							
3/8/2000							
3/9/2000							
3/10/2000					960		
3/11/2000	7.5	-310		34.8	6	1220	50
3/12/2000	7.45	-320		34.6		1280	

Table B-2. TNT acclimated sludge solids data for anaerobic reactor 5 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)	
3/13/2000							
3/14/2000							
3/15/2000							
3/16/2000							
3/17/2000	7.5	-310		34.2	2040		
3/18/2000	7.55	-310		34.7	6	1340	50
3/19/2000	7.1	-315		34.4		1260	
3/20/2000	7.2	-305		34.4		1200	
3/21/2000							
3/22/2000							
3/23/2000							
3/24/2000	7.35	-310		34.6		840	
3/25/2000	7.5	-305		34.5	6	1440	50
3/26/2000	7.05	-310		34.9		1380	
3/27/2000	7.3	-315		34.8		1160	
3/28/2000							
3/29/2000							
3/30/2000							
3/31/2000					680		
4/1/2000	7.5	-305		35	6	1240	50
4/2/2000	7.05	-315		34.6		1320	
4/3/2000	7.3	-310		34.2		1200	
4/4/2000							
4/5/2000							
4/6/2000	7.5	-305		34.7		540	
4/7/2000	7.6	-315		34.3		180	
4/8/2000	7.55	-315		34.2	6	860	100 -500 mL sludge
4/9/2000	7.1	-310		34.6		1260	
4/10/2000	7.2	-315		34.9		1180	
4/11/2000							
4/12/2000							
4/13/2000							
4/14/2000						1260	
4/15/2000	7.45	-320		34.6	6	880	100
4/16/2000	6.75	-315		35.1		1060	
4/17/2000	6.9	-315		34		1160	
4/18/2000	7	-310		34.8		780	
4/19/2000	7.05	-310		34.6		300	
4/20/2000							
4/21/2000	7.1	-315		35		340	
4/22/2000	7.5	-315		35.6	6	1000	50
4/23/2000	7.1	-320		34.9		1140	
4/24/2000	7.2	-310		34.7		1260	
4/25/2000	7.35	-320		34.6		680	
4/26/2000	7.45	-325		34.9		240	
4/27/2000	7.5	-315		34.2		160	
4/28/2000	7.55	-315		33.7		180	

Table B-2. TNT acclimated sludge solids data for anaerobic reactor 5 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)
4/29/2000	7.55	-310		33.9	6	960
4/30/2000	7.15	-320		34.5		1280
5/1/2000	7.25	-315		34.3		1320
5/2/2000	7.4	-320		34.8		560
5/3/2000	7.45	-315		34.9		220
5/4/2000	7.45	-320		34.6		200
5/5/2000	7.55	-315		34.7		160
5/6/2000	7.1	-320		35.7	6	1040
5/7/2000	7.15	-320		35.2		1140
5/8/2000	7.3	-320		34.5		1180
5/9/2000	7.4	-320		34.2		620
5/10/2000	7.5	-325		34.6		260
5/11/2000	7.45	-320		34.1		180
5/12/2000	7.55	-315		34.7		200
5/13/2000	7.55	-315		35.3	6	1160
5/14/2000	7.05	-320		35.4		1380
5/15/2000	7.25	-320		35.5		1100
5/16/2000	7.4	-310		35.7		380
5/17/2000	7.45	-320		36.2		240
5/18/2000	7.45	-320		36.1		200
5/19/2000	7.55	-315		35.7		180
5/20/2000	7.6	-320	12	34.6	6	1240
5/21/2000	7.1	-320	12.2	34.7		1280
5/22/2000	7.25	-310	12	35.2		1200
5/23/2000	7.35	-315	11.9	35.3		460
5/24/2000	7.5	-320	12.3	35.6		200
5/25/2000	7.5	-320	12.44	35.3		220
5/26/2000	7.55	-310	12.6	35.5		160
5/27/2000	7.55	-320	12.7	35.7	6	1400
5/28/2000	7.15	-315	12.4	35.8		1280
5/29/2000	7.3	-320	12.7	35.2		1020
5/30/2000	7.35	-315	12.9	35.5		420
5/31/2000	7.45	-310	13.1	35.9		180
6/1/2000	7.45	-320	12.8	34		180
6/2/2000	7.55	-320	12.9	34.6		220
6/3/2000	7.5	-320	13.2	34.5	6	100

-500 mL sludge

Table B-3. TNT acclimated sludge solids data for anaerobic reactor 9.

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)	
10/21/1999	7.55	-315	10.4	34.5			
10/22/1999	7.6	-320	10.4	34.4			
10/23/1999	7.6	-320	10.6	34.5	6	100	-500mL sludge
10/24/1999	7.05	-315	9.5	34.7			
10/25/1999	7.2	-300	9.5	34.9			
10/26/1999	7.3	-305	9.6	35			
10/27/1999	7.35	-300	9.3	35.3			
10/28/1999	7.45	-305	9.5	35.6			
10/29/1999	7.45	-305	9.6	35.9			
10/30/1999	7.5	-300	9.6	35.5	6	100	
10/31/1999	7.05	-295	8.8	35.5			
11/1/1999	7.2	-300	9	35.6			
11/2/1999	7.3	-295	9.1	35.8			
11/3/1999	7.4	-295	9.4	35.6			
11/4/1999	7.4	-295	9.3	35.5			
11/5/1999	7.45	-305	9.5	35.3			
11/6/1999	7.45	-300	9.6	35	6	1160	50
11/7/1999	7.15	-305	9.7	35.2	1220		
11/8/1999							
11/9/1999	7.2	-305	9.9	35.4	1460		
11/10/1999	7.35	-310	9.8	35.3	380		
11/11/1999	7.4	-315	9.9	35.6	240		0.0515 g TNT I in 4mL MeOH
11/12/1999	7.4	-305	10	35.1	160		
11/13/1999	7.4	-310	9.9	35	6	1080	50
11/14/1999	7	-310	10	35	1280		
11/15/1999	7.1	-305	10.3	24.8	1040		
11/16/1999	7.2	-310	10.1	35.1	540		
11/17/1999	7.35	-300	10.3	35.6	300		
11/18/1999	7.4	-305	10.5	35.7	150		
11/19/1999	7.45	-315	10.5	35.1	140		
11/20/1999	7.5	-315	10.4	34.7	6	940	50
11/21/1999	7.05	-305	10.5	34.6	1260		
11/22/1999	7	-310	10.6	34.9	1140		
11/23/1999	6.95	-305	10.4	34.5	560		
11/24/1999	7.25	-310	10.5	34.7	280		
11/25/1999	7.35	-310	10.7	34.5	220		0.0511 g TNT in 4mL MeOH
11/26/1999			10.8		180		
11/27/1999	7.45	-295	10.6	35	6	1020	50
11/28/1999	6.95	-310	10.8	35.1	1320		
11/29/1999	7.3	-305	10.9	35.2	980		
11/30/1999	7.35	-310	11	35	600		
12/1/1999							
12/2/1999							
12/3/1999	7.35	-300	10.8	34.9	750		
12/4/1999	7.4	-300	11	35.4	6	1150	50
12/5/1999	7.05	-305	10.9	35.5	1050		
12/6/1999	7.1	-310	11	35.3	1450		
12/7/1999	7.2	-305	11.2	35.6	800		

Table B-3. TNT acclimated sludge solids data for anaerobic reactor 9 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)	
12/8/1999	7.3	-310	11.3	35.2	780		
12/9/1999	7.35	-310	11.1	34.7	460		0.0513 g TNT in 4mL MeOH
12/10/1999							
12/11/1999	7.4	-310	11.6	34.6	6	75	50
12/12/1999	7.2	-300	11.4	34.8		1180	
12/13/1999	7.35	-305	11.6	35.7		1120	
12/14/1999							
12/15/1999	7.4	-300	11.5	35.2		680	
12/16/1999	7.15	-310	11.8	34.9		1600	
12/17/1999	7.3	-310	11.7	35.4		620	
12/18/1999	7.4	-320	11.9	35.6	6	820	100
12/19/1999	6.95	-310	11.2	35.3		1160	-500 mL sludge
12/20/1999	7.1	-315	11.4	35.3		940	
12/21/1999	7.2	-305	11.1	35		800	
12/22/1999							
12/23/1999							
12/24/1999					740		
12/25/1999	7.45	-310		34.6	6	1020	100
12/26/1999							
12/27/1999	7.25	-315		34.9		2020	
12/28/1999	7.4	-310		35.1		820	
12/29/1999							
12/30/1999							
12/31/1999	7.55	-310		35.3		520	
1/1/2000	7.55	-310		35.9	6	1020	50
1/2/2000	7.05	-315		34.6		1240	
1/3/2000	7.15	-320		34.9		1080	
1/4/2000	7.3	-315		34.6		660	
1/5/2000	7.35	-310		34.8		300	
1/6/2000	7.45	-315		34.3		220	
1/7/2000	7.5	-320		34.4		180	
1/8/2000	7.55	-315		34.7	6	1120	50
1/9/2000	7	-320		34.6		1240	
1/10/2000	7.15	-315		34.1		960	
1/11/2000	7.25	-320		34.5		580	
1/12/2000	7.4	-315		34.8		220	
1/13/2000	7.45	-315		34.5		240	
1/14/2000						180	
1/15/2000	7.5	-320		34.7	6	980	50
1/16/2000	7	-315		34.8		1220	
1/17/2000	7.1	-320		34.6		1020	
1/18/2000	7.3	-320		34.3		820	
1/19/2000							
1/20/2000							
1/21/2000					640		
1/22/2000	7.55	-315		35.7	6	1140	50
1/23/2000	7.15	-310		35.5		1300	
1/24/2000	7.25	-310		35.8		1040	

Table B-3. TNT acclimated sludge solids data for anaerobic reactor 9 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)
1/25/2000						
1/26/2000						
1/27/2000						
1/28/2000					1280	
1/29/2000	7.55	-320	35.7	6	1240	50
1/30/2000	7.1	-315	36.4		1200	
1/31/2000	7.2	-305	36.8		1180	
2/1/2000	7.35	-320	35.4		420	
2/2/2000	7.45	-310	35.1		260	
2/3/2000	7.55	-315	34.9		160	
2/4/2000	7.55	-310	34.6		180	
2/5/2000	7.5	-310	34.5	6	1420	50
2/6/2000	7	-310	34.7		1160	
2/7/2000	7.1	-315	33.3		1060	
2/8/2000	7.2	-315	33.6		440	
2/9/2000	7.3	-305	34.2		340	
2/10/2000	7.45	-310	34.7		180	
2/11/2000	7.5	-310	34.6		160	
2/12/2000	7.5	-315	35.5	6	1020	100
2/13/2000	6.95	-305	34.9		1140	-500 mL sludge
2/14/2000	7.05	-310	35		1260	
2/15/2000						
2/16/2000						
2/17/2000						
2/18/2000					980	
2/19/2000	7.45	-310	34.5	6	920	100
2/20/2000	7	-315	34.4		1220	
2/21/2000	7.1	-305	35.3		1160	
2/22/2000	7.2	-310	35.6		740	
2/23/2000						
2/24/2000						
2/25/2000					520	
2/26/2000	7.5	-320	34.7	6	1020	50
2/27/2000	7.05	-315	34.5		1340	
2/28/2000	7.15	-315	34.8		1160	
2/29/2000	7.25	-310	34.1		580	
3/1/2000						
3/2/2000						
3/3/2000					500	
3/4/2000	7.5	-320	35.7	6	1180	50
3/5/2000	7.05	-325	35.5		1340	
3/6/2000	7.15	-310	35.5		1180	
3/7/2000						
3/8/2000						
3/9/2000						
3/10/2000					1040	
3/11/2000	7.55	-315	35.9	6	1140	50
3/12/2000	7.15	-315	35.4		1260	

Table B-3. TNT acclimated sludge solids data for anaerobic reactor 9 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)	
3/13/2000							
3/14/2000							
3/15/2000							
3/16/2000							
3/17/2000	7.55	-315		35.5	2080		
3/18/2000	7	-310		35.4	6	1220	50
3/19/2000	7.1	-310		34.5		1380	
3/20/2000	7.2	-305		33.7		1240	
3/21/2000							
3/22/2000							
3/23/2000							
3/24/2000	7.5	-310		35.7	800		
3/25/2000	7.55	-310		34.5	6	1280	50
3/26/2000	7.1	-315		34.1		1340	
3/27/2000	7.25	-315		33.7		1300	
3/28/2000							
3/29/2000							
3/30/2000							
3/31/2000					780		
4/1/2000	7.45	-310		33.9	6	1340	50
4/2/2000	7	-305		35.2		1320	
4/3/2000	7.2	-310		34.4		1160	
4/4/2000							
4/5/2000							
4/6/2000	7.45	-310		34.8		680	
4/7/2000	7.55	-315		34.1		200	
4/8/2000	7.55	-315		34.6	6	940	100
4/9/2000	7.1	-310		34.9		1320	-500 mL sludge
4/10/2000	7.25	-305		34.8		1000	
4/11/2000							
4/12/2000							
4/13/2000							
4/14/2000					1440		
4/15/2000	7.4	-315		34.3	6	800	100
4/16/2000	6.9	-310		34.7		1120	
4/17/2000	7	-315		34.5		1240	
4/18/2000	7.1	-320		33.1		600	
4/19/2000	7.15	-320		33.7		360	
4/20/2000							
4/21/2000	7.3	-320		34.4		460	
4/22/2000	7.55	-315		34.2	6	980	50
4/23/2000	7.1	-315		33.9		1200	
4/24/2000	7.2	-320		33.8		1320	
4/25/2000	7.25	-315		34.1		740	
4/26/2000	7.4	-320		34		220	
4/27/2000	7.45	-310		34.6		160	
4/28/2000	7.55	-320		35.3		160	

Table B-3. TNT acclimated sludge solids data for anaerobic reactor 9 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)
4/29/2000	7.5	-320		35.4	6	940
4/30/2000	7.05	-325		35.7		1240
5/1/2000	7.2	-320		35.8		1380
5/2/2000	7.3	-310		35		640
5/3/2000	7.4	-320		35.4		200
5/4/2000	7.5	-320		34.7		200
5/5/2000	7.5	-320		34.4		160
5/6/2000	7.45	-320		35.3	6	1120
5/7/2000	7	-320		35.1		1220
5/8/2000	7.1	-315		35		1000
5/9/2000	7.35	-325		34.8		720
5/10/2000	7.4	-320		34.7		260
5/11/2000	7.5	-320		35.5		160
5/12/2000	7.45	-310		35.8		160
5/13/2000	7.45	-320		35.6	6	1100
5/14/2000	7.05	-320		35.4		1260
5/15/2000	7.2	-315		36.7		1180
5/16/2000	7.3	-320		36.3		480
5/17/2000	7.45	-320		36.1		220
5/18/2000	7.45	-315		35.9		180
5/19/2000	7.5	-315		35.7		160
5/20/2000	7.5	-320	12.2	35.2	6	1260
5/21/2000	7.05	-320	12.5	35.4		1180
5/22/2000	7.25	-320	12.4	35.6		1200
5/23/2000	7.35	-315	12.3	35.4		620
5/24/2000	7.4	-315	13.5	35.3		220
5/25/2000	7.5	-320	12.5	35.5		200
5/26/2000	7.5	-315	12.5	35.6		200
5/27/2000	7.55	-315	12.6	35.2	6	1360
5/28/2000	7.1	-315	12.8	35.5		1380
5/29/2000	7.3	-320	12.8	35.7		980
5/30/2000	7.4	-315	12.6	35.6		520
5/31/2000	7.5	-310	12.8	35.4		260
6/1/2000	7.5	-315	12.9	35.2		200
6/2/2000	7.5	-315	12.7	35.4		180
6/3/2000	7.55	-310	12.9	35.6	6	100

-500 mL sludge

Table B-4. Non-acclimated sludge solids data for anaerobic reactor 2.

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)	
10/21/1999	7.6	-320	9.6	35.4			
10/22/1999	7.55	-315	9.5	35.5			
10/23/1999	7.6	-320	9.6	35.4	6	100	-500mL sludge
10/24/1999	7.05	-315	8.7	36.3			
10/25/1999	7.25	-300	8.8	35.7			
10/26/1999	7.3	-310	9	35.5			
10/27/1999	7.4	-305	9.2	35.2			
10/28/1999	7.45	-305	9.4	35.3			
10/29/1999	7.45	-300	9.3	34.8			
10/30/1999	7.45	-295	9.3	35.3	6	100	
10/31/1999	7.05	-290	8.6	35.2			
11/1/1999	7.2	-300	8.7	35.2			
11/2/1999	7.3	-305	8.8	35.5			
11/3/1999	7.3	-300	8.6	35.8			
11/4/1999	7.4	-305	8.9	36.1			
11/5/1999	7.5	-295	9	33.6			
11/6/1999	7.45	-300	9.2	34.1	6	980	50
11/7/1999	7.1	-305	9.1	35		1440	
11/8/1999						1080	
11/9/1999	7.3	-305	9.3	35.1		540	
11/10/1999	7.35	-310	9.2	35.2		220	
11/11/1999	7.4	-315	9.5	35.2		160	4 mL MeOH
11/12/1999	7.45	-305	9.3	35.3		140	
11/13/1999	7.45	-310	9.4	35.5	6	1280	50
11/14/1999	7.1	-300	9.5	35.3		1100	
11/15/1999	7.2	-300	9.6	35.4		960	
11/16/1999	7.35	-310	9.7	35.2		620	
11/17/1999	7.45	-310	9.8	35.2		360	
11/18/1999	7.45	-320	9.6	35		200	
11/19/1999	7.5	-305	9.9	35.1		120	
11/20/1999	7.5	-300	9.9	34.6	6	1300	50
11/21/1999	7.15	-310	9.6	35.1		1250	
11/22/1999	7.2	-315	9.8	35.3		920	
11/23/1999	7.25	-315	9.9	34.9		420	
11/24/1999	7.35	-305	10	34.8		280	
11/25/1999	7.4	-305	10.2	34.4		320	4 mL MeOH
11/26/1999						100	
11/27/1999	7.4	-310	10	34.6	6	850	50
11/28/1999	7.05	-305	10.3	34.7		1180	
11/29/1999	7.15	-310	10.1	34.8		1100	
11/30/1999	7.2	-310	10.4	34.9		700	
12/1/1999							
12/2/1999							
12/3/1999	7.35	-300	10.4	34.8		800	
12/4/1999	7.5	-300	10.2	34.8	6	1350	50
12/5/1999	7.2	-305	10.3	34.7		1100	
12/6/1999	7.3	-310	10.3	35		1200	
12/7/1999	7.3	-310	10.2	35.1		550	

Table B-4. Non-acclimated sludge solids data for anaerobic reactor 2 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)	
12/8/1999	7.35	-305	10.5	35.3	350		4 mL MeOH
12/9/1999	7.35	-310	10.6	35.2	200		
12/10/1999						4750	
12/11/1999	7.5	-300	10.5	35	6	920	50
12/12/1999	7.2	-310	10.6	34.5		1200	
12/13/1999	7.35	-310	10.7	34.6		1120	
12/14/1999							
12/15/1999	7.5	-315	10.9	34.8		740	
12/16/1999	7.25	-305	10.7	35.2		320	
12/17/1999	7.35	-310	11	35.3		120	
12/18/1999	7.45	-315	10.9	35.9	6	840	100
12/19/1999	7.05	-320	10	35.6		1180	-500 mL sludge
12/20/1999	7.25	-315	10.2	35.1		960	
12/21/1999	7.4	-310	10.3	35.4		680	
12/22/1999							
12/23/1999							
12/24/1999					780		
12/25/1999	7.5	-310		35.3	6	920	100
12/26/1999							
12/27/1999	7.2	-320		35.5		2060	
12/28/1999	7.35	-320		35.4		720	
12/29/1999							
12/30/1999							
12/31/1999	7.5	-320		35.3		900	
1/1/2000	7.5	-315		35	6	880	50
1/2/2000	7.05	-320		35.8		1320	
1/3/2000	7.15	-320		35.7		1160	
1/4/2000	7.25	-315		35		400	
1/5/2000	7.35	-315		35.5		240	
1/6/2000	7.45	-310		35.4		160	
1/7/2000	7.5	-310		34.6		200	
1/8/2000	7.55	-315		34.1	6	1140	50
1/9/2000	7.1	-310		34.4		1040	
1/10/2000	7.2	-325		34.7		1160	
1/11/2000	7.35	-315		35.2		580	
1/12/2000	7.45	-315		35.1		260	
1/13/2000	7.5	-320		35.3		200	
1/14/2000						220	
1/15/2000	7.5	-320		35.7	6	920	50
1/16/2000	7.05	-320		35.5		1160	
1/17/2000	7.2	-315		35.9		1040	
1/18/2000	7.3	-315		36.1		700	
1/19/2000							
1/20/2000							
1/21/2000					720		
1/22/2000	7.55	-315		35.7	6	1020	50
1/23/2000	7.1	-310		35.4		1380	
1/24/2000	7.2	-320		34.6		1140	

Table B-4. Non-acclimated sludge solids data for anaerobic reactor 2 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)
1/25/2000						
1/26/2000						
1/27/2000						
1/28/2000					1140	
1/29/2000	7.4	-310	35.4	6	1200	50
1/30/2000	7	-305	35.3		1240	
1/31/2000	7.1	-305	35.7		940	
2/1/2000	7.2	-310	35		660	
2/2/2000	7.4	-315	34.6		320	
2/3/2000	7.5	-315	34.3		200	
2/4/2000	7.5	-310	34.5		220	
2/5/2000	7.5	-315	34.8	6	1140	50
2/6/2000	7.05	-310	35.7		1280	
2/7/2000	7.15	-305	35.4		1140	
2/8/2000	7.3	-310	35.3		580	
2/9/2000	7.45	-315	34.9		360	
2/10/2000	7.55	-310	34.1		160	
2/11/2000	7.55	-305	34.6		200	
2/12/2000	7.5	-300	33.9	6	920	100
2/13/2000	7	-310	33.7		1140	-500 mL sludge
2/14/2000	7.1	-295	34.5		1360	
2/15/2000						
2/16/2000						
2/17/2000						
2/18/2000					1100	
2/19/2000	7.4	-320	34.7	6	1000	100
2/20/2000	6.9	-320	34.5		1360	
2/21/2000	7.05	-310	35.7		960	
2/22/2000	7.15	-305	34.8		640	
2/23/2000						
2/24/2000						
2/25/2000					580	
2/26/2000	7.45	-320	35.5	6	960	50
2/27/2000	7	-315	35.3		1280	
2/28/2000	7.1	-305	36.1		1240	
2/29/2000	7.2	-310	36.5		520	
3/1/2000						
3/2/2000						
3/3/2000					640	
3/4/2000	7.55	-320	36.4	6	1040	50
3/5/2000	7.1	-315	35.4		1340	
3/6/2000	7.2	-315	34.3		1240	
3/7/2000						
3/8/2000						
3/9/2000						
3/10/2000					1020	
3/11/2000	7.55	-310	34.6	6	1180	50
3/12/2000	7.1	-315	34.7		1340	

Table B-4. Non-acclimated sludge solids data for anaerobic reactor 2 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)
3/13/2000						
3/14/2000						
3/15/2000						
3/16/2000						
3/17/2000	7.5	-305	36.2		1980	
3/18/2000	7.55	-315	35.3	6	1200	50
3/19/2000	7.05	-310	35.8		1340	
3/20/2000	7.2	-310	35.9		960	
3/21/2000						
3/22/2000						
3/23/2000						
3/24/2000	7.5	-310	35.1		1020	
3/25/2000	7.5	-315	34.9	6	1320	50
3/26/2000	7	-315	34.6		1440	
3/27/2000	7.1	-305	36.3		940	
3/28/2000						
3/29/2000						
3/30/2000						
3/31/2000					860	
4/1/2000	7.1	-305	29.4	6	1280	50
4/2/2000	7.2	-310			1320	
4/3/2000	7.3	-310	29.2		1260	
4/4/2000						
4/5/2000						
4/6/2000	7.5	-305	35.1		620	
4/7/2000	7.55	-310	35.4		180	
4/8/2000	7.55	-310	34.7	6	920	100
4/9/2000	7.05	-315	34.2		1220	
4/10/2000	7.2	-315	33.9		1300	
4/11/2000						
4/12/2000						
4/13/2000						
4/14/2000					1180	
4/15/2000	7.45	-320	35.2	6	1000	100
4/16/2000	7.2	-320	34.6		1120	
4/17/2000	7.45	-315	34.1		1060	
4/18/2000	7.1	-320	34.5		620	
4/19/2000	7.35	-320	34.3		400	
4/20/2000						
4/21/2000	6.7	-320	34.2		480	
4/22/2000	7.45	-315	34.6	6	1080	50
4/23/2000	7	-315	34.7		1260	
4/24/2000	7.15	-320	35.2		1240	
4/25/2000	7.2	-315	35.7		580	
4/26/2000	7.35	-310	35.8		240	
4/27/2000	7.45	-315	36		160	
4/28/2000	7.45	-320	36		180	

Table B-4. Non-acclimated sludge solids data for anaerobic reactor 2 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)
4/29/2000	7.5	-315		35.8	6	1120
4/30/2000	7.05	-320		35.5		1200
5/1/2000	7.2	-320		34.7		1180
5/2/2000	7.35	-325		34.8		660
5/3/2000	7.45	-320		33.9		280
5/4/2000	7.5	-320		34.2		180
5/5/2000	7.45	-310		34.6		200
5/6/2000	7.5	-320		35.7	6	1040
5/7/2000	7.1	-315		35.3		1260
5/8/2000	7.15	-320		35.4		1080
5/9/2000	7.25	-320		34.5		640
5/10/2000	7.4	-315		35.1		220
5/11/2000	7.4	-315		35		140
5/12/2000	7.5	-320		34.8		180
5/13/2000	7.45	-320		34.7	6	960
5/14/2000	6.95	-310		34.5		1340
5/15/2000	7	-325		34.6		1220
5/16/2000	7.2	-320		34.5		540
5/17/2000	7.35	-320		34.8		180
5/18/2000	7.4	-315		34.7		160
5/19/2000	7.45	-320	12.2	35		200
5/20/2000	7.5	-315	12.4	35	6	1220
5/21/2000	7	-315	12.3	35.1		1260
5/22/2000	7.1	-325	12.5	35.2		1140
5/23/2000	7.2	-320	12.3	35.1		500
5/24/2000	7.3	-320	12.5	35		320
5/25/2000	7.45	-315	12.4	34.7		180
5/26/2000	7.55	-320	12.5	34.5		160
5/27/2000	7.55	-320	12.9	34.3	6	1440
5/28/2000	7.15	-315	12.6	34.7		1320
5/29/2000	7.25	-320	12.6	34.5		920
5/30/2000	7.35	-320	12.9	34.3		420
5/31/2000	7.45	-320	13.3	34.8		220
6/1/2000	7.55	-320	13.4	35.9		200
6/2/2000	7.6	-315	13.4	35.7		220
6/3/2000	7.6	-320	13.2	35.3	6	100

-500 mL sludge

Table B-5. Non-acclimated sludge solids data for anaerobic reactor 3.

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)	
10/21/1999	7.5	-310	10.5	35.6			
10/22/1999	7.55	-320	10.4	35.3			
10/23/1999	7.55	-320	10.5	35.2	6	100	-500mL sludge
10/24/1999	7	-315	9.3	35.9			
10/25/1999	7.15	-295	9.4	35.3			
10/26/1999	7.2	-300	9.2	35.4			
10/27/1999	7.35	-310	9.4	35.7			
10/28/1999	7.45	-305	9.5	32.7			
10/29/1999	7.45	-305	9.6	31.1			
10/30/1999	7.4	-300	9	25.5	6	100	
10/31/1999	7.1	-300	9.3	33.2			
11/1/1999	7.15	-295	9.1	34.1			
11/2/1999	7.25	-300	9.4	34.5			
11/3/1999	7.4	-8	9.2	34.5			
11/4/1999	7.45	-305	9.4	34.8			
11/5/1999	7.45	-300	9.2	35.2			
11/6/1999	7.45	-300	9.5	35.5	6	1000	50
11/7/1999	7.05	-305	9.4	35.3		980	
11/8/1999						1360	
11/9/1999	7.3	-310	9.6	35.1		360	
11/10/1999	7.3	-305	9.5	35.2		140	
11/11/1999	7.4	-300	9.7	35.2		180	4 mL MeOH
11/12/1999	7.45	-310	9.7	35		150	
11/13/1999	7.45	-300	9.8	35	6	1240	50
11/14/1999	7.05	-300	10	34.9		1060	
11/15/1999	7.15	-310	9.8	34.9		1100	
11/16/1999	7.3	-305	10.2	34.6		250	
11/17/1999	7.35	-300	10.3	34.6		200	
11/18/1999	7.4	-305	10.1	34.8		200	
11/19/1999	7.45	-310	10.4	34.9		120	
11/20/1999	7.05	-305	10.5	35	6	1200	50
11/21/1999	7.15	-305	10.4	35.3		1100	
11/22/1999	7.2	-310	10.6	35.4		1060	
11/23/1999	7.3	-315	10.5	35.1		850	
11/24/1999	7.35	-310	10.7	34.9		700	
11/25/1999	7.45	-310	10.9	35.9		320	4 mL MeOH
11/26/1999						140	
11/27/1999	7.4	-310	10.9	34.6	6	850	50
11/28/1999	7.1	-305	10.7	34.7		1400	
11/29/1999	7.2	-310	11	35		1100	
11/30/1999	7.3	-315	11.3	34.9		700	
12/1/1999							
12/2/1999							
12/3/1999	7.35	-315	11.1	34.8		620	
12/4/1999	7.5	-305	11.5	35.3	6	1140	50
12/5/1999	7.1	-305	11.3	35.1		1100	
12/6/1999	7.3	-310	11.3	35		1200	
12/7/1999	7.35	-305	11.4	34.7		440	

Table B-5. Non-acclimated sludge solids data for anaerobic reactor 3 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)	
12/8/1999	7.4	-310	10.3	34.6	350		4 mL MeOH
12/9/1999	7.45	-310	11.4	34.8	150		
12/10/1999							
12/11/1999	7.5	-305	11.7	34.9	6	860	50
12/12/1999	7.15	-300	11.5	35.3		1420	
12/13/1999	7.25	-305	11.8	36.1		1040	
12/14/1999							
12/15/1999	7.3	-305	11.5	35.8		880	
12/16/1999	6.95	-300	11.7	35.6		1600	
12/17/1999	7.05	-305	11.8	35.2		860	
12/18/1999	7.4	-310	10.9	35.3	6	820	100
12/19/1999	7.1	-310	11.1	35.3		1140	-500 mL sludge
12/20/1999	7.2	-315	10.9	35		1120	
12/21/1999	7.35	-300	11.3	34.6		640	
12/22/1999							
12/23/1999							
12/24/1999					840		
12/25/1999	7.45	-310		34.2	6	880	100
12/26/1999							
12/27/1999	7.15	-320		35.6		2100	
12/28/1999	7.25	-315		35.2		780	
12/29/1999							
12/30/1999							
12/31/1999	7.45	-315		35.6		760	
1/1/2000	7.45	-320		35.9	6	1040	50
1/2/2000	7.05	-320		36.1		1180	
1/3/2000	7.15	-320		35.4		1120	
1/4/2000	7.25	-315		35.8		500	
1/5/2000	7.35	-315		35.5		320	
1/6/2000	7.45	-320		35.2		160	
1/7/2000	7.5	-320		35		180	
1/8/2000	7.5	-315		34.9	6	20	50
1/9/2000	7	-305		34.7		1100	
1/10/2000	7.1	-310		34.9		1500	
1/11/2000	7.2	-320		35.3		380	
1/12/2000	7.3	-320		34.8		1040	
1/13/2000	7.45	-315		35.7		1800	
1/14/2000							
1/15/2000	7.45	-320		35.5	6	1060	50
1/16/2000	7	-320		35.5		1280	
1/17/2000	7.1	-315		35.9		960	
1/18/2000	7.2	-320		34.1		660	
1/19/2000							
1/20/2000							
1/21/2000					620		
1/22/2000	7.5	-305		35.5	6	1200	50
1/23/2000	7.1	-310		35.9		1260	
1/24/2000	7.2	-320		36.2		1120	

Table B-5. Non-acclimated sludge solids data for anaerobic reactor 3 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)	
1/25/2000							
1/26/2000							
1/27/2000							
1/28/2000					1220		
1/29/2000	7.5	-320	35.4	6	1180	50	
1/30/2000	7.05	-305	35.7		1340		
1/31/2000	7.15	-305	34.1		1040		
2/1/2000	7.25	-315	34.7		660		
2/2/2000	7.4	-305	34.8		280		
2/3/2000	7.5	-310	35.6		160		
2/4/2000							
2/5/2000	7.5	-310	35.3	6	1280	50	
2/6/2000	7.05	-310	35.4		1220		
2/7/2000	7.25	-315	35.9		1060		
2/8/2000	7.35	-315	36.4		660		
2/9/2000	7.5	-310	36.2		280		
2/10/2000	7.6	-305	35.7		220		
2/11/2000	7.6	-320	35.5		240		
2/12/2000	7.6	-305	35.1	6	960	100	-500 mL sludge
2/13/2000	7.05	-310	34.6		1060		
2/14/2000	7.2	-310	34.3		1260		
2/15/2000							
2/16/2000							
2/17/2000							
2/18/2000					1240		
2/19/2000	7.45	-320	35.3	6	980	100	
2/20/2000	6.95	-310	35		1300		
2/21/2000	7.05	-315	34.8		1060		
2/22/2000	7.2	-310	34.4		680		
2/23/2000							
2/24/2000							
2/25/2000					440		
2/26/2000	7.5	-315	36.3	6	1000	50	
2/27/2000	7	-315	35.6		1220		
2/28/2000	7.05	-305	35.4		1160		
2/29/2000	7.15	-310	35.7		560		
3/1/2000							
3/2/2000							
3/3/2000					680		
3/4/2000	7.5	-315	34.6	6	1160	50	
3/5/2000	7.05	-315	34.8		1240		
3/6/2000	7.2	-310	35.7		1160		
3/7/2000							
3/8/2000							
3/9/2000							
3/10/2000					980		
3/11/2000	7.45	-320	34.1	6	1120	50	
3/12/2000	7	-315	34.9		1540		

Table B-5. Non-acclimated sludge solids data for anaerobic reactor 3 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)	
3/13/2000							
3/14/2000							
3/15/2000							
3/16/2000							
3/17/2000	7.45		35.1		2000		
3/18/2000	7.5		35.8	6	1160	50	
3/19/2000	7		36.3		1380		
3/20/2000	7.1		35.6		1140		
3/21/2000							
3/22/2000							
3/23/2000							
3/24/2000	7.55		34.2		940		
3/25/2000	7.55		34.5	6	1280	50	
3/26/2000	7.05		34.3		1360		
3/27/2000	7.15		33.9		1140		
3/28/2000							
3/29/2000							
3/30/2000							
3/31/2000					840		
4/1/2000	7.05		35.6	6	1340	50	
4/2/2000					1400		
4/3/2000	7.25	-310	34.1		1060		
4/4/2000							
4/5/2000							
4/6/2000	7.5	-315	34.3		700		
4/7/2000	7.55	-315	33.1		140		
4/8/2000	7.5	-310	33.6	6	960	100	-500 mL sludge
4/9/2000	7.1	-315	34.4		1180		
4/10/2000	7.2	-305	34.7		1300		
4/11/2000							
4/12/2000							
4/13/2000							
4/14/2000					1260		
4/15/2000	7.45	-315	34.5	6	1040	100	
4/16/2000	7.15	-320	34.8		1120		
4/17/2000	7.2	-320	35.8		1180		
4/18/2000	7.3	-315	35.6		660		
4/19/2000	7.45	-320	34.7		360		
4/20/2000							
4/21/2000	7.5	-315	34.3		380		
4/22/2000	7.5	-315	34.6	6	1060	50	
4/23/2000	7.05	-320	34.9		1100		
4/24/2000	7.25	-315	35		1240		
4/25/2000	7.35	-320	35		580		
4/26/2000	7.4	-320	34.6		320		
4/27/2000	7.45	-315	34.4		260		
4/28/2000	7.45	-320	34.4		180		

Table B-5. Non-acclimated sludge solids data for anaerobic reactor 3 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)	
4/29/2000	7.5	-315		35.6	6	1000	50
4/30/2000	7.1	-315		35.9		1220	
5/1/2000	7.2	-320		36.2		1160	
5/2/2000	7.3	-320		36.1		620	
5/3/2000	7.35	-315		35.8		240	
5/4/2000	7.5	-310		33.6		200	
5/5/2000	7.55	-310		29.1		220	
5/6/2000	7.45	-315		32.7	6	940	50
5/7/2000	7.05	-325		33.4		1160	
5/8/2000	7.1	-320		34.1		1260	
5/9/2000	7.2	-320		35.3		740	
5/10/2000	7.35	-320		35.5		260	
5/11/2000	7.45	-315		35.9		160	
5/12/2000	7.45	-320		34.6		160	
5/13/2000	7.5	-320		34.3	6	1060	50
5/14/2000	7	-320		34.1		1280	
5/15/2000	7.25	-315		34.6		1120	
5/16/2000	7.25	-315		34.6		480	
5/17/2000	7.4	-320		34.2		240	
5/18/2000	7.45	-320		34.8		220	
5/19/2000	7.55	-315	12.2	33.9		160	
5/20/2000	7.55	-315	12.2	35.2	6	1140	50
5/21/2000	7.05	-310	12	35.3		1340	
5/22/2000	7.2	-315	12.2	35.5		1180	
5/23/2000	7.3	-320	12.3	35.6		440	
5/24/2000	7.35	-325	12.3	35.4		260	
5/25/2000	7.5	-310	12.5	36.1		160	
5/26/2000	7.5	-320	12.4	35.7		160	
5/27/2000	7.55	-315	12.6	35.4	6	1380	50
5/28/2000	7.1	-310	12.5	34.9		1340	
5/29/2000	7.25	-320	12.6	34.6		1040	
5/30/2000	7.3	-315	12.9	34.4		460	
5/31/2000	7.45	-315	12.6	34.6		200	
6/1/2000	7.5	-320	12.9	34.7		160	
6/2/2000	7.55	-320	13.3	35.3		240	
6/3/2000	7.5	-315	13.2	35.3	6		100 -500 mL sludge

Table B-6. Non-acclimated sludge solids data for anaerobic reactor 8.

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)	
10/21/1999	7.45	-320	10	36.1			
10/22/1999	7.5	-320	9.8	35.6			
10/23/1999	7.45	-315	10.2	35.3	6	100	-500mL sludge
10/24/1999	7.05	-310	9.3	35.4			
10/25/1999	7.2	-310	9	35.2			
10/26/1999	7.25	-300	9.1	35.7			
10/27/1999	7.4	-310	9	35.8			
10/28/1999	7.4	-310	9.1	35.5			
10/29/1999	7.5	-305	9.1	35.2			
10/30/1999	7.45	-300	9.2	35.5	6	100	
10/31/1999	7.1	-295	8.6	35.7			
11/1/1999	7.25	-300	8.7	35.5			
11/2/1999	7.4	-300	8.6	35.2			
11/3/1999	7.5	-395	8.9	35.1			
11/4/1999	7.45	-305	8.7	34.9			
11/5/1999	7.45	-305	8.9	25			
11/6/1999	7.5	-300	9	35.1	6	860	50
11/7/1999	7.2	-305	9.2	35.3		1260	
11/8/1999			8.3			1020	
11/9/1999	7.25	-300	9	35		360	
11/10/1999	7.3	-300	9.1	35.2		250	
11/11/1999	7.35	-310	9.3	35.3		200	4 mL MeOH
11/12/1999	7.45	-310	9.3	34.9		160	
11/13/1999	7.5	-305	9.4	35.9	6	40	50
11/14/1999	7.1	-305	9.5	35.5		1100	
11/15/1999	7.15	-300	9.6	35.3		1050	
11/16/1999	7.3	-310	9.5	34.9		350	
11/17/1999	7.4	-295	9.6	34.8		200	
11/18/1999	7.45	-305	9.7	35.2		220	
11/19/1999	7.45	-305	9.7	35.5		100	
11/20/1999	7.5	-305	9.8	34.9	6	860	50
11/21/1999	7.05	-300	9.9	34.8		1280	
11/22/1999	7.25	-310	10.1	34.6		1200	
11/23/1999	7.3	-305	10	34.9		420	
11/24/1999	7.35	-305	10.2	35.4		220	
11/25/1999	7.4	-315	10.5	35.7		140	4 mL MeOH
11/26/1999						100	
11/27/1999	7.35	-310	10.4	35.3	6	900	50
11/28/1999	7.1	-310	10.4	35		1200	
11/29/1999	7.3	-320	10.3	34.7		1000	
11/30/1999	7.35	-315	10.6	34.8		560	
12/1/1999							
12/2/1999							
12/3/1999	7.35	-315	10.6	35		440	
12/4/1999	7.5	-305	10.5	35.1	6	1200	50
12/5/1999	7.1	-310	10.6	35.7		1220	
12/6/1999	7.2	-310	10.5	35.9		1000	
12/7/1999	7.3	-305	10.6	35.5		550	

Table B-6. Non-acclimated sludge solids data for anaerobic reactor 8 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)	
12/8/1999	7.35	-310	10.5	35.1	240		4 mL MeOH
12/9/1999	7.45	-310	10.7	35	180		
12/10/1999							
12/11/1999	7.5	-310	11	35	6	780	50
12/12/1999	7.05	-305	11.2	34.7		1380	
12/13/1999	7.2	-315	11.3	34.6		1060	
12/14/1999							
12/15/1999	7.3	-310	11.4	34.8		760	
12/16/1999	7.4	-300	11.3	35		200	
12/17/1999	7.45	-305	11.2	35.7		160	
12/18/1999	7.45	-300	10.4	35.1	6	940	100
12/19/1999	7.1	-300	10.2	35.1		1040	-500 mL sludge
12/20/1999	7.35	-310	10.5	35.3		1060	
12/21/1999	7.25	-300	10.6	34.9		600	
12/22/1999							
12/23/1999							
12/24/1999					740		
12/25/1999	7.45	-320		34.4	6	940	100
12/26/1999							
12/27/1999	7.25	-320		35.5		1940	
12/28/1999	7.35	-320		35.1		720	
12/29/1999							
12/30/1999							
12/31/1999	7.45	-310		35.2		1000	
1/1/2000	7.45	-320		35	6	960	50
1/2/2000	7	-320		34.9		1220	
1/3/2000	7.1	-300		34.6		1160	
1/4/2000	7.2	-315		34.8		480	
1/5/2000	7.35	-310		34.5		220	
1/6/2000	7.45	-315		34.8		180	
1/7/2000	7.5	-320		35.6		200	
1/8/2000	7.55	-320		36.8	6	1180	50
1/9/2000	7	-315		36.3		1120	
1/10/2000	7.15	-315		35.7		1080	
1/11/2000	7.25	-320		34.9		760	
1/12/2000	7.35	-315		34		240	
1/13/2000	7.4	-315		34.4		200	
1/14/2000						160	
1/15/2000	7.55	-320		35.1	6	1100	50
1/16/2000	7.05	-320		34.2		1260	
1/17/2000	7.15	-315		34.7		1020	
1/18/2000	7.2	-305		34.5		540	
1/19/2000							
1/20/2000							
1/21/2000					580		
1/22/2000	7.5	-310		33.8	6	1120	50
1/23/2000	7.1	-310		34.4		1300	
1/24/2000	7.2	-320		34.6		1160	

Table B-6. Non-acclimated sludge solids data for anaerobic reactor 8 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)	
1/25/2000							
1/26/2000							
1/27/2000							
1/28/2000					1180		
1/29/2000	7.5	-320	36	6	1260	50	
1/30/2000	7	-315	34.5		1160		
1/31/2000	7.1	-315	34.2		1100		
2/1/2000	7.25	-310	34.1		720		
2/2/2000	7.4	-320	34.5		280		
2/3/2000	7.5	-315	35.2		200		
2/4/2000							
2/5/2000	7.55	-315	34.8	6	1340	50	
2/6/2000	7.1	-310	32.5		1100		
2/7/2000	7.2	-320	33.6		940		
2/8/2000	7.3	-315	34.2		740		
2/9/2000	7.4	-310	35.5		360		
2/10/2000	7.55	-315	35.6		220		
2/11/2000	7.6	-310	34.6		120		
2/12/2000	7.55	-320	35.3	6	840	100	-500 mL sludge
2/13/2000	7.05	-310	35.6		1120		
2/14/2000	7.15	-315	35.8		1360		
2/15/2000							
2/16/2000							
2/17/2000							
2/18/2000					1120		
2/19/2000	7.4	-315	35.9	6	940	100	
2/20/2000	6.95	-320	35.2		1220		
2/21/2000	7.1	-310	35.5		1140		
2/22/2000	7.2	-310	35.8		540		
2/23/2000							
2/24/2000							
2/25/2000					660		
2/26/2000	7.45	-315	35.6	6	1080	50	
2/27/2000	7	-315	35.7		1160		
2/28/2000	7.1	-310	35.2		1240		
2/29/2000	7.25	-320	34.5		520		
3/1/2000							
3/2/2000							
3/3/2000					580		
3/4/2000	7.5	-320	34.8	6	1060	50	
3/5/2000	7	-320	34.1		1360		
3/6/2000	7.15	-310	34.5		1180		
3/7/2000							
3/8/2000							
3/9/2000							
3/10/2000					1080		
3/11/2000	7.5	-320	34.7	6	1040	50	
3/12/2000	7	-315	34.4		1340		

Table B-6. Non-acclimated sludge solids data for anaerobic reactor 8 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)	
3/13/2000							
3/14/2000							
3/15/2000							
3/16/2000							
3/17/2000	7.55		35.9		2060		
3/18/2000	7.55		36.2	6	1240	50	
3/19/2000	7.1		35.3		1380		
3/20/2000	7.3		34.7		1040		
3/21/2000							
3/22/2000							
3/23/2000							
3/24/2000	7.4		34.8		980		
3/25/2000	7.55		33.4	6	1380	50	
3/26/2000	7		34		1360		
3/27/2000	7.15		35.2		1240		
3/28/2000							
3/29/2000							
3/30/2000							
3/31/2000					640		
4/1/2000	6.9	-315	33.8	6	1380	50	
4/2/2000					1300		
4/3/2000	7.15	-315	34.9		1280		
4/4/2000							
4/5/2000							
4/6/2000	7.25	-310	35		520		
4/7/2000	6.85	-315	35.1		200		
4/8/2000	7.6	-310	35.8	6	740	100	-500 mL sludge
4/9/2000	7.15	-310	36.5		1320		
4/10/2000	7.2	-310	34.6		1220		
4/11/2000							
4/12/2000							
4/13/2000							
4/14/2000					1360		
4/15/2000	7.4	-320	35.8	6	920	100	
4/16/2000	7	-320	35.4		1280		
4/17/2000	7.15	-315	34.4		1220		
4/18/2000	7.25	-320	33.7		620		
4/19/2000	7.35	-315	33.9		340		
4/20/2000							
4/21/2000	7.45	-310	34.4		360		
4/22/2000	7.45	-320	35.1	6	940	50	
4/23/2000	7	-315	35.3		1080		
4/24/2000	7.15	-315	35.6		1260		
4/25/2000	7.25	-320	35.2		640		
4/26/2000	7.35	-325	35.6		320		
4/27/2000	7.4	-320	35.9		200		
4/28/2000	7.4	-320	35.7		240		

Table B-6. Non-acclimated sludge solids data for anaerobic reactor 8 (continued).

Date	pH	Eh (mV)	Cond (mho)	Food (g)	Gas (mL)	DI (mL)
4/29/2000	7.45	-325		35.3	6	1040
4/30/2000	7.1	-310		34.6		50
5/1/2000	7.2	-320		34.9		1160
5/2/2000	7.25	-315		35.2		600
5/3/2000	7.35	-320		34.7		260
5/4/2000	7.45	-320		34.8		160
5/5/2000	7.45	-320		34.6		160
5/6/2000	7.45	-315		34.6	6	900
5/7/2000	7.05	-315		34.8		1200
5/8/2000	7.2	-320		34.1		1160
5/9/2000	7.3	-320		34.5		660
5/10/2000	7.4	-315		35.2		340
5/11/2000	7.45	-310		35.3		140
5/12/2000	7.5	-315		35.3		160
5/13/2000	7.45	-320		35.5	6	940
5/14/2000	7.05	-320		35.7		1160
5/15/2000	7.15	-310		35.6		1240
5/16/2000	7.25	-320		35.7		540
5/17/2000	7.4	-320		35.9		280
5/18/2000	7.5	-320		35.5		220
5/19/2000	7.4	-315	12.4	35.6		180
5/20/2000	7.5	-315	12.3	35.4	6	1080
5/21/2000	7.1	-320	12.4	35.4		1280
5/22/2000	7.2	-315	12.6	35.6		1140
5/23/2000	7.35	-320	12.5	35.8		520
5/24/2000	7.4	-320	12.6	35.1		240
5/25/2000	7.5	-315	12.9	35.3		180
5/26/2000	7.55	-320	12.9	35.5		200
5/27/2000	7.55	-315	13	35.6	6	1400
5/28/2000	7.15	-320	13	35.5		1260
5/29/2000	7.3	-320	12.9	35.6		940
5/30/2000	7.4	-315	13.1	35.3		580
5/31/2000	7.5	-315	13.2	35.2		160
6/1/2000	7.55	-310	13.1	34.8		180
6/2/2000	7.55	-320	13.5	34.9		220
6/3/2000	7.6	-320	13.2	35.1	6	100
						-500 mL sludge

Table B-7. RDX activated sludge reactor data

Time (hr)	Sample Conc. (mg/L) NO2-	Dilution	Original Conc. (mg/L) NO2
Hydrolysate	0.2367	60606	14346.20
0	0.2393	2000	478.68
4	0.1157	2000	231.45
21.61667	0.0046	400	1.84
24	0.4996	1000	499.55
28	0.2321	1000	232.10
47	0.0120	1000	12.00
48	0.5130	1000	513.00
53	0.2650	1000	265.00
71.66667	0.0660	100	6.60
72	0.5470	1000	547.00
79	0.1540	1000	154.00
89.66667	0.1300	100	13.00
105.5	0.0670	100	6.70
105.5833	0.4900	1000	490.00
120.75	0.0860	100	8.60
120.8333	0.4620	1000	462.00
144.75	0.0480	100	4.80
144.8333	0.4930	1000	493.00
167.25	0.0910	100	9.10
184.25	0.0290	100	2.90
192	0.4890	1000	489.00
215.9167	0.0350	100	3.50
215.9333	0.5070	1000	507.00
239.9167	0.0270	100	2.70
240	0.4720	1000	472.00
269	0.2350	10	2.35
269.25	0.4890	1000	489.00
286.8333	0.1150	10	1.15
311.9667	0.4930	1000	493.00
360.1	0.3830	10	3.83
360.1667	0.5990	1000	599.00
416.9167	0.0900	100	9.00
451	0.4720	1000	472.00
475.6667	0.1100	10	1.10
476.4333	0.4790	1000	479.00
477.4333	0.3900	1000	390.00
478.4333	0.3000	1000	300.00
479.4333	0.2480	1000	248.00
480.4333	0.1520	1000	152.00
481.4333	0.0670	1000	67.00
482.4333	0.0013	1000	1.30
499.0333	0.0070	1000	7.00
499.25	0.5230	1000	523.00
500.25	0.4090	1000	409.00

Table B-7. RDX activated sludge reactor data (continued).

Time (hr)	Sample Conc. (mg/L) NO ₂ -	Dilution	Original Conc. (mg/L) NO ₂
504.25	0.0410	1000	41.00
505.25	0.0170	1000	17.00
506.25	0.0140	1000	14.00
507.25	0.0280	100	2.80
527	0.4780	1000	478.00
553.6667	0.1470	100	14.70
554.6667	0.1470	100	1.93
559.25	0.5030	1000	503.00
563.5	0.0680	100	6.80
980.75	0.0900	10	0.90
980.8333	0.5220	1000	522.00
1006.5	0.4990	1000	499.00
1031.367	0.4250	1000	425.00
1076.833	0.2430	1000	243.00

Table B-7. HMX activated sludge reactor data.

Time (hr)	Sample Conc. (mg/L) NO ₂ -	Dilution	Original Conc. (mg/L) NO ₂
0	0.3980	1000	398.00
6	0.4040	1000	404.00
24.66667	0.0620	100	6.20
25	0.4470	1000	447.00
32	0.0390	1000	39.00
54.66667	0.1300	10	1.30
58.5	0.4620	1000	462.00
73.75	0.0460	100	4.60
73.83333	0.4670	1000	467.00
97.75	0.3800	100	38.00
97.83333	0.4930	1000	493.00
120.25	0.0420	100	4.20
137.25	0.0370	100	3.70
145	0.4890	1000	489.00
168.9333	0.0980	10	0.98
192.9167	0.4630	1000	463.00
222	0.1160	20	2.32
222.25	0.4690	1000	469.00
239.8333	0.4820	10	4.82
264.9667	0.4550	1000	455.00
312.9333	0.2510	10	2.51
313	0.4770	1000	477.00
336.9167	0.1690	20	3.38

Table B-7. HMX activated sludge reactor data.

Time (hr)	Sample Conc. (mg/L) NO ₂ -	Dilution	Original Conc. (mg/L) NO ₂
428.6667	0.1360	10	1.36
475.75	0.0920	10	0.92
476	0.5080	1000	508.00
477	0.4460	1000	446.00
478	0.3820	1000	382.00
479	0.3210	1000	321.00
480	0.2600	1000	260.00
481	0.2070	1000	207.00
482	0.1640	1000	164.00
483	0.1140	1000	114.00
484	0.0640	1000	64.00
485	0.3070	100	30.70
504.9167	0.1130	10	1.13
507	0.4530	1000	453.00
528.8	0.0490	100	4.90
529.0167	0.4490	1000	449.00
530.25	0.0350	100	3.50
547	0.2930	10	2.93
547.0833	0.4740	1000	474.00
571.5	0.2220	10	2.22
571.5833	0.4860	1000	486.00
598.8833	0.1660	10	1.66

APPENDIX C
BMP ASSAY GAS DATA

Table C-1. Gas production from BMP assays receiving non-acclimated sludge solids and treatment with TNT.

Day #	1	2	3	4	6	8	10	14	20	26	32	38
Reactor 1	0.5	1	1.5	2	2.5	3.5	5	7.5	10	12.5	17	21.5
Reactor 2	0.5	1	1	1.5	2.5	3.5	5.5	7	9.5	13.5	19	23
Reactor 3	1	1.5	2	2	2.5	3	4.5	6.5	9	12.5	17	20.5
Average	0.67	1.17	1.5	1.8	2.5	3.3	5	7	9.5	12.8	17.7	21.7

Day #	44	50	56	62	68	74	82	90	Average	Std. Dev
Reactor 1	24	29	34.5	39.5	45	50.5	56.5	64	3.77	0.22
Reactor 2	26.5	29.5	35	42	46.5	50	54.5	61		
Reactor 3	25.5	29	34.5	39.5	44	48.5	53	56.5		
Average	25.3	29.2	34.7	40.3	45.2	49.7	54.7	60.5		

Table C-2. Gas production from BMP assays receiving TNT-acclimated sludge solids and treatment with TNT.

Day #	1	2	3	4	6	8	10	14	20	26	32	38
Reactor 1	0.5	0.5	1	1.5	2.5	3.5	5	7	9.5	11.5	15	21
Reactor 2	1	1	1.5	2	2.5	3.5	4.5	6	8	10.5	13.5	19
Reactor 3	0.5	1	1.5	2	3	4.5	5.5	8	10	12.5	14.5	18
Average	0.7	0.8	1.3	1.8	2.7	3.8	5	7	9.2	11.5	14.3	19.3

Day #	44	50	56	62	68	74	82	90	Average	Std. Dev
Reactor 1	24.5	30	35.5	41	47.5	52.5	61	67.5	4.80	0.23
Reactor 2	23	28.5	34.5	41	46.5	52	57.5	61.5		
Reactor 3	21.5	27	32.5	38.5	44	50.5	56	58		
Average	23	28.5	34.17	40.17	46	51.67	58.17	62.3		

Table C-3. Gas production from BMP assays receiving TNT-acclimated sludge solids and treatment with RDX.

Day #	1	2	3	4	6	8	10	12	14	16	18	20
Reactor 1	3.5	8.5	12	18.5	29	35.5	44	50.5	58	65	70.5	74
Reactor 2	3	7.5	10.5	16.5	28.5	36	42.5	48.5	54	61	68.5	74.5
Reactor 3	3	8	11.5	17	28.5	34.5	40.5	47	52.5	58.5	63.5	68
Average	3.2	8	11.3	17.3	28.7	35.3	42.3	48.7	54.8	61.5	67.5	72.2

Day #	22	24	26	28	30	32	34	36	38	40	42
Reactor 1	79.5	85.5	89.5	95	101	106	112.5	117.5	122.5	128	134
Reactor 2	80.5	86	91	96	101.5	106	111.5	116.5	121.5	126	130
Reactor 3	72.5	78	82.5	86	91.5	97	101.5	106	109.5	115	119.5
Average	77.5	83.2	87.7	92.3	98	103	108.5	113.3	117.8	123	127.8

Table C-3. Gas production from BMP assays receiving TNT-acclimated sludge solids and treatment with RDX (continued).

Day #	44	46	48	50	52	54	56	58	60	62	64
Reactor 1	141	144	155.5	161.5	167.5	174.5	181	188.5	196	203.5	209
Reactor 2	136.5	142.5	149	155.5	161	168.5	175	182.5	189.5	196	203.5
Reactor 3	126	131.5	136	140	146	152	158.5	164.5	170	177	182.5
Average	134.5	139.33	146.83	152.33	158.2	165	171.5	178.5	185.2	192.2	198.3

Day #	66	68	70	74	78	82	86	90	St. Dev	V ₉₀	t ₉₀
Reactor 1	218.5	227	232.5	246	257.5	269	273.5	275.5		247.0	74.7
Reactor 2	210.5	217	224.5	237.5	252	257	261.5	263.5		237.2	73.9
Reactor 3	188.5	196.5	207	219.5	242	259.5	264	267		240.3	77.7
Average	205.8	213.5	221.3	234.3	250.5	261.8	266.3	268.7	6.2	241.8	75.8

Table C-4. Gas production from BMP assays receiving non-acclimated sludge solids and treatment with RDX.

Day #	1	2	3	4	6	8	10	12	14	16	18	20
Reactor 1	3	8	11	17.5	26	35	43.5	52	57.5	62.5	66.5	70.5
Reactor 2	3.5	9	12.5	18	29	36.5	45.5	54	60.5	69.5	77.5	81
Reactor 3	3	7.5	11	16.5	28	37.5	47	56	63.5	70.5	73	77.5
Average	3.2	8.2	11.5	17.3	27.7	36.3	45.3	54	60.5	67.5	72.3	76.3

Day #	22	24	26	28	30	32	34	36	38	40	42
Reactor 1	75.5	80	85	89.5	94.5	99	104.5	109	114	118.5	125
Reactor 2	86	90.5	96	102.5	106.5	113.5	119.5	125.5	132	138	143.5
Reactor 3	83	88	94	98.5	103	109	113.5	118	122	129	135
Average	81.5	86.167	91.667	96.833	101.33	107.16	112.5	117.5	122.66	128.5	134.5
					3	7			7		

Day #	44	46	48	50	52	54	56	58	60	62	64
Reactor 1	133	139.5	147.5	155	161	167	174.5	183	188.5	196	205
Reactor 2	149	153	161	168.5	175	182.5	189	193.5	201	210.5	221
Reactor 3	141	147	155	162.5	169.5	175.5	182	189.5	198	205	213
Average	141	146.5	154.5	162	168.5	175	181.8	188.7	195.8	203.8	213

Day #	66	68	70	74	78	82	86	90	St Dev	V ₉₀	t ₉₀
Reactor 1	214.5	222	229.5	243	257	265	268.5	270		243	74
Reactor 2	233	241.5	249	258.5	264	270.5	273	274		246.6	68.0
Reactor 3	220.5	228.5	238	247	255	263.5	266	267		240.3	71.0
Average	222.7	230.7	238.8	249.5	258.7	266.3	269.2	270.3	3.5	243.3	71.7

Table C-5. Gas production from BMP assays receiving TNT-acclimated sludge solids and treatment with HMX.

Day #	1	2	3	4	6	8	10	12	14	16	18
Reactor 1	4	10	18.5	32	56.5	75.5	89.5	97	105.5	112	119.5
Reactor 2	3.5	9	17	31	51.5	69	85.5	93.5	101	108.5	116
Reactor 3	4	10	19	33.5	54	72.5	86	95.5	104.5	111	119.5
Average	3.8	9.7	18.2	32.2	54	72.3	87	95.3	103.7	110.5	118.3

Day #	20	22	24	26	28	30	32	34	36	38	40
Reactor 1	127	135.5	143	150.5	158	167.5	174	181.5	187	194.5	206
Reactor 2	124.5	132	139.5	146	152	157.5	165	175.5	188.5	200.5	214
Reactor 3	128.5	137	144.5	151	159	166.5	172.5	179	184	189	203.5
Average	126.7	134.8	142.3	149.2	156.3	163.8	170.5	178.7	186.5	194.67	207.8

Day #	42	44	46	48	50	52	54	56	62	68	74
Reactor 1	217.5	226	238	244.5	249	255	259.5	263	268	271	273.5
Reactor 2	225.5	234	245	252	257	262	265.5	268	270.5	272	276
Reactor 3	216	224.5	236	242.5	247.5	253	257	261.5	263	264.5	265.5
Average	219.7	228.2	239.7	246.3	251.2	256.2	260.7	264.2	267.2	269.2	271.7

Day #	82	90	St. Dev	V ₉₀	t ₉₀
Reactor 1	274.5	275		247.5	48.9
Reactor 2	277.5	278.5		250.7	47.6
Reactor 3	266.5	267.5		240.8	47.5
Average	272.8	273.7	5.6	246.3	48.0

Table C-6. Gas production from BMP assays receiving non-acclimated sludge solids and treatment with HMX.

Day #	1	2	3	4	6	8	10	12	14	16	18
Reactor 1	4.5	9.5	17	31.5	52	70.5	85	94.5	102	108.5	116.5
Reactor 2	4	9	17.5	32	51.5	69	84	92.5	101	109	117.5
Reactor 3	4	10.5	18	33.5	54.5	73	86.5	95	101.5	107	114.5
Average	4.2	9.7	17.5	32.3	52.7	70.8	85.2	94	101.5	108.2	116.2

Day #	20	22	24	26	28	30	32	34	36	38	40
Reactor 1	125	133	140.5	147.5	153	159	164	172	182.5	195	206.5
Reactor 2	127	135.5	143	150.5	158	164.5	173	182.5	193.5	204	213.5
Reactor 3	122.5	129	136	143.5	149.5	156	164.5	175.5	186	198.5	211
Average	124.8	132.5	139.8	147.2	153.5	159.8	167.2	176.7	187.3	199.2	210.3

Day #	42	44	46	48	50	52	54	56	62	68	74
Reactor 1	217	228	236.5	243.5	249	253	256	259	265.5	267.5	269.5
Reactor 2	224	236.5	245.5	255.5	260	263	266	269.5	272	273.5	274.5
Reactor 3	223.5	234.5	245	254	263.5	269	273	274.5	275.5	278	279
Average	221.5	233	242.3	251	257.5	261.7	265	267.7	271	273	274.3

Table C-6. Gas production from BMP assays receiving non-acclimated sludge solids and treatment with HMX (continued).

Day #	82	90	St. Dev	V ₉₀	t ₉₀
Reactor 1	270.5	271		243.9	48.1
Reactor 2	275	276.5		248.9	46.7
Reactor 3	279.5	280		252	47.6
Average	275	275.8	4.5	248.3	47.4

Table C-7. Gas production from BMP assays receiving non-acclimated sludge solids and treatment with denitrified RDX hydrolysate.

Day #	1	2	3	4	6	8	10	12	14	16	18
Reactor 1	10	20	43	74.5	130.5	167.5	200.5	219	233.5	247	254
Reactor 2	9	21	45.5	81	134	171.5	204	220.5	232	242.5	249
Reactor 3	9.5	19	39.5	69.5	123.5	162.5	191	215	229	238.5	246
Average	9.5	20	42.7	75	129.3	167.2	198.5	218.2	231.5	242.7	249.7

Day #	20	22	24	26	28	30	32	38	44	50
Reactor 1	256.5	261	263	265	267	268.5	269.5	271	272	273
Reactor 2	253.5	257	260.5	263	265	267.5	270	271.5	273	274
Reactor 3	248.5	252	255	258	259.5	261	262.5	265	266.5	268
Average	252.8	256.7	259.5	262	263.8	265.7	267.3	269.2	270.5	271.7

Day #	56	62	68	74	82	90	St. Dev	V ₉₀	t ₉₀
Reactor 1	274	275	276	277	277.5	278.5		250.7	15.3
Reactor 2	275.5	276.5	277.5	278.5	279.5	280		252	19.3
Reactor 3	269	270.5	271.5	273	274	275		247.5	19.2
Average	272.8	274	275	276.27	277	277.8	2.56	250.15	18.2

Table C-8. Gas production from BMP assays receiving non-acclimated sludge solids and treatment with raw RDX hydrolysate.

Day #	1	2	3	4	6	8	10	12	14	16	18	20
Reactor 1	9.5	20.5	22	23	23	24.5	26.5	28	31.5	36	40.5	46
Reactor 2	9	19	20	22	23	25.5	29	30.5	33	37.5	42.5	48.5
Reactor 3	8.5	18.5	19.5	21	23	25.5	27.5	30.5	35	39.5	44	50.5
Average	9	19.333	20.5	22	23	25.167	27.667	29.667	33.167	37.667	42.333	48.333

Day #	22	24	26	28	30	32	34	36	38	40	42
Reactor 1	52.5	59.5	67.5	77	91	114.5	135.5	154	178	199.5	221
Reactor 2	56.5	64	71.5	86.5	105	122	147	162.5	181.5	207	228.5
Reactor 3	59	66.5	75	91.5	115.5	133	158.5	179	195	213	232.5
Average	56	63.3	71.3	85	103.8	123.2	147	165.2	184.8	206.5	227.3

Table C-8. Gas production from BMP assays receiving non-acclimated sludge solids and treatment with raw RDX hydrolysate (continued).

Day #	44	50	56	62	68	74	82	90	St. Dev	V ₉₀	t ₉₀
Reactor 1	244.5	270	278.5	283	285.5	286.5	289	291		261.9	45.0
Reactor 2	242.5	261	273.5	278.5	281	281.5	282	283		254.7	45.7
Reactor 3	249	269.5	277.5	281.5	283.5	284	285	285.5		257.0	45.7
Average	245.3	266.8	276.5	281	283.3	284	285.3	286.5	4.1	257.9	45.4

Table C-9. Gas production from BMP assays receiving non-acclimated sludge solids and treatment with denitrified HMX hydrolysate.

Day #	1	2	3	4	6	8	10	12	14	16	18
Reactor 1	9.5	18.5	42	71	121	163.5	194.5	215	235	243	252.5
Reactor 2	10.5	22	49.5	78.5	128.5	167.5	199.5	219.5	235	245.5	253
Reactor 3	9.5	22.5	50.5	76.5	126	168.5	191	217	227.5	239	244.5
Average	9.833	21	47.333	75.333	125.167	166.5	195	217.167	232.5	242.5	250

Day #	20	22	24	26	28	30	32	38	44
Reactor 1	258	264	266.5	268.5	269.5	270	272	275.5	277.5
Reactor 2	255	259.5	262.5	263.5	264	264.5	265.5	268	270
Reactor 3	248	252.5	255	258.5	259.5	259.5	261	265	268
Average	253.7	258.7	261.3	263.5	264.3	264.7	266.2	269.5	271.8

Day #	50	56	62	68	74	82	90	St. Dev	V ₉₀	t ₉₀
Reactor 1	278	280	281.5	282.5	284	285	286		257.4	16.7
Reactor 2	272.5	275	276.5	278	279	280	281		252.9	17.6
Reactor 3	270	271.5	272.5	273	274	275.5	276.5		248.9	19.1
Average	273.5	275.5	276.8	277.8	279	280.2	281.2	4.8	253.1	17.8

Table C-10. Gas production from BMP assays receiving non-acclimated sludge solids and treatment with raw HMX hydrolysate.

Day #	1	2	3	4	6	8	10	12	14	16	18
Reactor 1	10.5	21	21	21.5	23	29.5	34.5	42.5	56	72	86.5
Reactor 2	9.5	19.5	20	20.5	22	27	32.5	39.5	52.5	68	82.5
Reactor 3	10.5	20.5	21.5	22	23.5	31	35.5	44	58.5	74	88
Average	10.2	20.3	20.8	21.3	22.8	29.2	34.2	42	55.7	71.3	85.7

Day #	20	22	24	26	28	30	32	34	36	38
Reactor 1	99.5	121.5	137	168.5	195	227	245	258.5	263	270
Reactor 2	96	112	125.5	157	186.5	214.5	228.5	239	247.5	254.5
Reactor 3	102.5	123.5	139	170.5	197.5	226	239.5	249	259.5	265.5
Average	99.3	119	133.8	165.3	193	222.5	237.7	248.8	256.7	263.3

Table C-10. Gas production from BMP assays receiving non-acclimated sludge solids and treatment with raw HMX hydrolysate (continued).

Day #	40	42	44	50	56	62	68	74	82	90
Reactor 1	274.5	276	279.5	282	283	283.5	284.5	285	286.5	287.5
Reactor 2	261	267.5	270.5	275.5	277.5	278.5	280	281	282	283.5
Reactor 3	271	275	278.5	282	283.5	283	285.5	287	288	289.5
Average	268.8	272.8	276.2	279.8	281.3	281.7	283.3	284.3	285.5	286.8

Day #	St. Dev	V ₉₀	t ₉₀
Reactor 1		258.8	34.8
Reactor 2		255.2	38.2
Reactor 3		260.6	36.4
Average	3.1	258.2	36.4

Table C-11. Gas production from BMP assays receiving non-acclimated sludge solids and no explosive additions.

Day #	1	2	3	4	6	8	10	12	14	16
React 1	9.5	19.5	45	80.5	134	172	196	211	227.5	242
React 2	10.5	19.5	47.5	79	135.5	173	199.5	218.5	234.5	247
React 3	10	20	45.5	74.5	127	163.5	183	212.5	225	235.5
Average	10	19.7	46	78	132.2	169.5	192.8	214	231	241.5

Day #	18	20	22	24	26	28	30	32	38	44
React 1	246	251.5	255	259	262.5	263.5	265	266	271	272
React 2	254.5	261.5	264	266.5	268	269	270	271.5	274	275
React 3	245	251.5	256	259.5	261	262.5	263.5	265	267	268
Average	248.5	254.8	258.3	261.7	263.8	265	266.2	267.5	270.7	271.7

Day #	50	56	62	68	74	82	90	St. Dev	V ₉₀	t ₉₀
React 1	273	273.5	274	274	274.5	274.5	275		247.5	18.8
React 2	276.5	277.5	278	279	279.5	280.5	281		252.9	17.6
React 3	268.5	269.5	270.5	271	272	272.5	273.5		246.2	18.2
Average	272.7	273.5	274.2	274.7	275.3	275.8	276.5	4.0	248.9	18.1

Table C-12. Gas production from BMP assays receiving TNT-acclimated sludge solids and no explosive additions.

Day #	1	2	3	4	6	8	10	12	14	16
React 1	9	20	47.5	80	132.5	168	192	206.5	225	237.5
React 2	10	20.5	49	82	139.5	163	184	200.5	218	233
React 3	8.5	18.5	44.5	76	129.5	164.5	189	207	226.5	241
Average	9.167	19.7	47	79.3	133.8	165.2	188.3	204.7	223.2	237.2

Day #	18	20	22	24	26	28	30	32	38	44
React 1	244	250	255.5	259	262.5	264.5	266	267	268	269
React 2	241.5	248	253	257	259	261.5	263	264.5	265.5	266
React 3	246.5	254	258	262	266	267.5	269	271	272	273
Average	244	250.7	255.5	259.3	262.5	264.5	266	267.5	268.5	269.3

Day #	50	56	62	68	74	82	90	St. Dev	V ₉₀	t ₉₀
React 1	269.5	270.5	271	272	272.5	273	274		246.6	18.8
React 2	267	267.5	268.5	269	270	270.5	271		243.9	18.6
React 3	274	274.5	275.5	276.5	277	277.5	278.5		250.7	19.5
Average	270.2	270.8	271.6	272.5	273.2	273.7	274.5	3.8	247.1	18.9

APPENDIX D
HIGH ANAEROBIC SOLIDS ASSAY DATA

Table D-1. High anaerobic solids data for TNT treatment of Reactor 1 containing TNT acclimated sludge solids.

Time (min)	Calculated Concentration (ppm)					Conc. Transformed (ppm)		
	TNT	Unk	4ADNT	2ADNT	Total	TNT	Unk	ADNT
1	71.13	1.27	1.83	0.83	75.05	0.00	-1.27	-3.92
5	49.37	11.71	4.20	2.74	68.02	21.75	10.05	3.11
10	35.24	14.65	7.43	5.70	63.02	35.89	21.24	8.11
30	11.69	20.37	13.56	10.58	56.20	59.43	39.07	14.93
60	0.84	28.05	13.95	10.92	53.76	70.28	42.23	17.37
90	-0.04	16.97	14.67	11.62	43.22	71.17	54.20	27.91
120	-0.04	1.12	9.87	6.61	17.55	71.17	70.05	53.58
150	0.01	0.45	6.27	3.06	9.79	71.11	70.67	61.33
180	0.08	0.52	5.98	2.62	9.20	71.04	70.53	61.92
210	0.00	0.01	1.65	0.13	1.80	71.12	71.11	69.33

Table D-2. High anaerobic solids data for TNT treatment of Reactor 2 containing TNT acclimated sludge solids.

Time (min)	Calculated Concentration (ppm)					Conc. Transformed (ppm)		
	TNT	Unk	4ADNT	2ADNT	Total	TNT	Unk	ADNT
1	67.63	1.98	2.81	1.42	73.85	3.49	1.52	-2.72
60	-0.04	12.42	14.35	11.36	38.09	71.17	58.75	33.04
90	6.20	18.75	4.21	3.27	32.43	64.92	46.17	38.70

Table D-3. High anaerobic solids data for TNT treatment of Reactor 3 containing TNT acclimated sludge solids.

Time (min)	Calculated Concentration (ppm)					Conc. Transformed (ppm)		
	TNT	Unk	4ADNT	2ADNT	Total	TNT	Unk	ADNT
1	66.05	1.09	2.82	1.75	71.71	5.08	3.99	-0.59
30	12.78	19.36	14.05	10.72	56.91	58.35	38.99	14.22
60	0.18	4.17	14.54	10.84	29.73	70.94	66.78	41.39

Table D-4. High anaerobic solids data for TNT treatment of HAS Reactor 1 containing non-acclimated sludge solids.

Time (min)	Calculated Concentration (ppm)						Conc. Transformed (ppm)		
	TNT	Unk	4ADNT	2ADNT	Total ADNT	Total	Unk	ADNT	ADNT
1	74.7	0.0	1.9	1.4	3.3	77.9	8.1	8.2	4.9
5	66.2	0.5	5.0	5.4	10.4	77.1	16.6	16.1	5.7
10	60.9	1.8	7.0	7.5	14.5	77.2	21.9	20.1	5.6
30	38.8	10.0	10.2	11.6	21.7	70.6	44.0	34.0	12.2
120	23.0	5.6	15.0	19.4	34.4	63.0	59.8	54.3	19.8
160	17.3	6.0	17.2	22.8	40.0	63.3	65.5	59.5	19.5
480	1.1	1.7	22.5	32.6	55.1	57.9	81.7	79.9	24.8
640	0.1	0.2	19.9	29.7	49.7	49.9	82.7	82.5	32.9
720	0.0	0.0	16.4	26.5	42.9	43.0	82.8	82.7	39.8
840	0.0	0.0	11.2	15.7	26.9	26.8	82.8	82.9	56.0
960	0.1	0.1	1.9	0.6	2.5	2.8	82.7	82.5	80.0

Table D-5. High anaerobic solids data for TNT treatment of HAS Reactor 2 containing non-acclimated sludge solids.

Time (min)	Calculated Concentration (ppm)						Conc. Transformed (ppm)		
	TNT	Unk	4ADNT	2ADNT	Total ADNT	Total	Unk	ADNT	ADNT
1	78.0	1.7	1.6	1.0	2.6	82.2	4.8	3.2	0.6
30	39.7	10.0	11.2	13.3	24.5	74.3	43.1	33.0	8.5
480	2.6	3.9	21.8	31.3	53.1	59.6	80.2	76.3	23.2

Table D-6. High anaerobic solids data for TNT treatment of HAS Reactor 2 containing non-acclimated sludge solids.

Time (min)	Calculated Concentration (ppm)						Conc. Transformed (ppm)		
	TNT	Unk	4ADNT	2ADNT	Total ADNT	Total	Unk	ADNT	ADNT
1	76.8	1.8	2.9	1.7	4.6	83.1	6.0	4.3	-0.3
30	40.8	3.2	9.6	12.1	21.7	65.7	42.0	38.8	17.0
480	4.7	3.1	16.2	33.2	49.5	57.3	78.1	75.0	25.5

Table D-7. Laboratory error replicates for TNT treatment.

Elapsed Time (min)	Calculated Concentration (ppm)					
	TNT	Unk	4ADNT	2ADNT	Total ADNT	Total
480	4.7	3.1	16.2	33.2	49.5	57.3
480	1.43	1.77	22.50	32.28	54.8	57.98
480	0.93	2.03	22.48	32.32	54.8	57.77
480	1.04	1.34	22.53	33.18	55.7	58.09
average	2.03	2.05	20.94	32.75	53.7	57.78
st dev	1.81	0.73	3.13	0.52	3.66	0.37

Table D-8. High anaerobic solids data for RDX treatment of HAS reactors containing TNT acclimated sludge solids.

Time (min)	Conc. (ppm)			Avg. (ppm)
	1	2	3	
1	83.7	83.7	94.7	87.4
90	74.9			74.9
360	54.6			54.6
540	34.8			34.8
960	9.4	9.2	16.3	11.6
1920	3.5		9.3	6.4

Table D-9. High anaerobic solids data for RDX treatment of HAS reactors containing non-acclimated sludge solids.

Time (min)	RDX Conc. (ppm)			Avg. (ppm)
	1	2	3	
1	93.9	103.0	104.9	100.6
90	91.1	93.2		92.2
180	81.2			81.2
360	65.3		72.2	68.8
540	40.2		38.8	39.5
960	12.4	12.9	13.1	12.8

Table D-10. High anaerobic solids data for RDX treatment background values.

	Background Concentration (ppm)
	2.7
	6.8
	6.3
	8.1
average	6.0
st dev	2.3

Table D11. Laboratory error replicates for RDX treatment.

	RDX Conc. (ppm)
	80.4
	80.8
	81.2
	82.3
Average	81
StDev	0.9128
	65

Table D12. Machine error replicates for RDX treatment.

	RDX Conc. (ppm)
	82.50 82.23 82.18
Average StDev	82 0.169

Table D-13. High anaerobic solids data for mixed explosive treatment of HAS reactors containing TNT-acclimated sludge solids.

Time (min)	Concentration							
	HMX (ppm)	RDX (ppm)	Unknown (ppm)	TNT (ppm)	4ADNT (ppm)	2ADNT (ppm)	ADNT (ppm)	Total (ppm)
1	43.70	27.67	2.27	11.08	3.84	2.12	5.96	19.31
10	54.11	24.63	3.05	0.47	4.90	2.16	7.06	10.57
20	50.05	22.83	2.99	-0.01	4.82	2.58	7.40	10.38
40	52.48	22.00	-0.08	0.34	0.64	0.08	0.72	0.98
60	83.25	22.84	-0.18	-0.18	0.49	0.08	0.57	0.21
90	52.16	20.84	-0.18	0.00	0.49	0.08	0.57	0.39
360	72.10	0.55	-0.18	0.00	0.49	0.08	0.57	0.39
2880	4.11	14.02	-0.18	0.00	0.49	0.08	0.57	0.39

Table D-14. High anaerobic solids data for mixed explosive treatment of HAS reactors containing non-acclimated sludge solids.

Time (min)	Concentration							
	HMX (ppm)	RDX (ppm)	TNT (ppm)	Unknown (ppm)	4ADNT (ppm)	2ADNT (ppm)	ADNT (ppm)	Total (ppm)
1	29.26	26.76	25.66	0.27	-0.04	0.49	0.45	26.38
5	28.37	25.10	14.00	1.96	1.96	3.67	5.63	21.59
10	24.96	27.44	8.48	4.77	2.93	6.93	9.86	23.11
20	26.90	24.26	4.49	5.48	3.45	9.23	12.68	22.65
30	25.98	25.55	1.23	5.66	3.98	8.87	12.86	19.75
180	24.31	22.35	-0.04	-0.04	-0.04	0.49	0.45	0.36
360	20.26	0.33	-0.04	-0.04	-0.04	0.49	0.45	0.36

APPENDIX E
RDX AND HMX HYDROLYSATE PREPARATION.

Table E-1. HMX hydrolysis notes as presented by Los Alamos National Laboratory personnel.

The HMX hydrolysate was prepared by LANL personnel using a standard hydrolysis procedure. Solid HMX, sodium hydroxide pellets, and water were added in the amounts listed below to a glass container. The mixture was heated to 80°C and held for approximately 4 hours to complete the hydrolysis of the HMX, yielding the hydrolysate solution. The solution was neutralized with hydrochloric acid prior to shipping.

HMX Base Hydrolysis (1-14-00)

Operator: Robert Bishop, Bishop@lanl.gov

Weight of HMX = 100.13 g

Weight of NaOH (pellets) = 60.12 g

Total amount of water was approximately 1300 mL

At a temperature of 80 C or above for approximately 4 hours

Amount of HCl used to neutralize was 28.97 g

Final pH was 6.9

Table E-2. RDX hydrolysis notes as presented by Los Alamos National Laboratory personnel.

The RDX hydrolysate was prepared by LANL personnel using a standard hydrolysis procedure. Solid RDX, sodium hydroxide pellets, and water were added in the amounts listed below to a glass container. The mixture was heated to 80°C and held for approximately 1 hour to complete the hydrolysis of the RDX, yielding the hydrolysate solution. The solution was neutralized with hydrochloric acid prior to shipping.

RDX Base Hydrolysis (1-19-00)

Operator: Robert Bishop, Bishop@lanl.gov

Weight of RDX = 100.35 g

Weight of NaOH (pellets) = 60.23 g

Total amount of water was approximately 1000 mL

At a temperature of 80 C or above for approximately 1 hr

Amount of HCl used to neutralize was 15.44 g

Final pH was 6.7

APPENDIX F
BMP STANDARD MEDIA PREPARATION

Solution	Compound	Concentration (g/l)
S1	Sample	<2 g/l in assay liquid
S2		
S3		
S4	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ NH_4Cl $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ KCl $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ H_3BO_3 $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ZnCl_2 $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ H_2WO_4	16.7 26.6 120 86.7 1.33 2 0.38 0.18 0.17 0.14 0.15 0.007
S5	$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	370
S6	$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$	500
S7	Biotin Folic acid Pyridoxine hydrochloride Riboflavin Thiamin Nicotinic acid Pantothenic acid B_{12} p-aminobenzoic acid Thioctic acid	0.002 0.002 0.02 0.005 0.005 0.005 0.005 0.0001 0.005 0.005

APPENDIX G
UNIVERSITY OF FLORIDA WASTEWATER TREATMENT PLANT SLUDGE
ANALYSIS

Table G-1. University of Florida Wastewater Treatment Plant sludge analysis for second Quarter of 1998

Sample date: 5/13/98

Parameter	Result	Units
pH	7.03	S.U.
Total Solids	0.671	%
Total Potassium	0.6	%
Arsenic	37.2	mg/kg
Cadmium	1.94	mg/kg
Chromium	23.2	mg/kg
Copper	411	mg/kg
Lead	36.1	mg/kg
Mercury	7.75	mg/kg
Molybdenum	16.1	mg/kg
Nickel	14.8	mg/kg
Selenium	29.8	mg/kg
Zinc	498	mg/kg
BOD	2.2	mg/L

Table G-2. University of Florida Wastewater Treatment Plant sludge analysis for second quarter of 1999

Sample date: 5/13/98

Parameter	Result	Units
pH	6.08	S.U.
Total Solids	2.67	%
Total Potassium	0.524	%
Arsenic	1.87	mg/kg
Cadmium	3.75	mg/kg
Chromium	14.2	mg/kg
Copper	315	mg/kg
Lead	24.0	mg/kg
Mercury	13.5	mg/kg
Molybdenum	10.5	mg/kg
Nickel	18.4	mg/kg
Selenium	9.36	mg/kg
Zinc	6.07	mg/kg
Total Nitrogen	0.36	%
Total Phosphorus	2.02	%

APPENDIX H
SAMPLE HPLC CHROMATOGRAMS

Sample Name :
FileName : D:\GEORGE\2000\DATC024.RAW
Method :
Start Time : 0.00 min
Scale Factor: 0.0

End Time : 60.00 min
Plot Offset: 291.6 mAU

Sample #: 24
Date : 4/25/01 09:08 PM
Time of Injection: 6/11/00 03:05 PM
Low Point : 290.51 mAU
High Point : 474.07 mAU
Plot Scale: 183.6 mAU

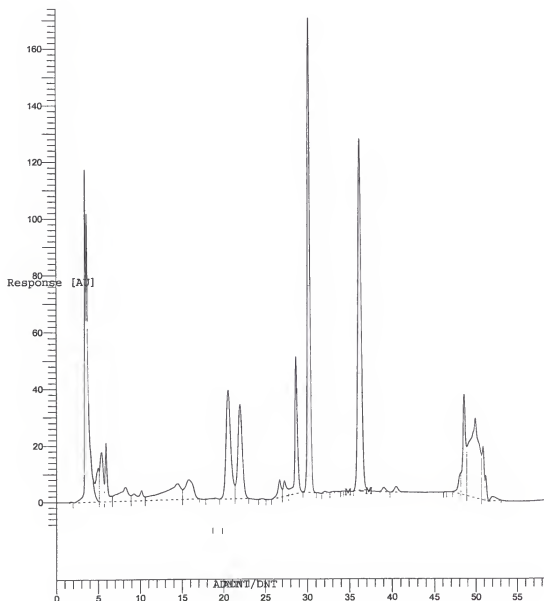


Figure H-1. HPLC Chromatogram showing TNT (36.14 min), 4ADNT (39.03 min), and 2ADNT (40.42 min), one minute after treatment of a non-acclimated sludge solids anaerobic reactor with 100 ppm TNT.

Sample Name :
FileName : D:\GEORGE\2000\DATC027.RAW
Method :
Start Time : 0.00 min End Time : 60.00 min
Scale Factor: 0.0 Plot Offset: 289 mAU

Sample #: 27 Page 1 of 1
Date : 4/25/01 09:12 PM
Time of Injection: 6/11/00 06:09 PM
Low Point : 288.70 mAU High Point : 492.65 mAU
Plot Scale: 203.9 mAU

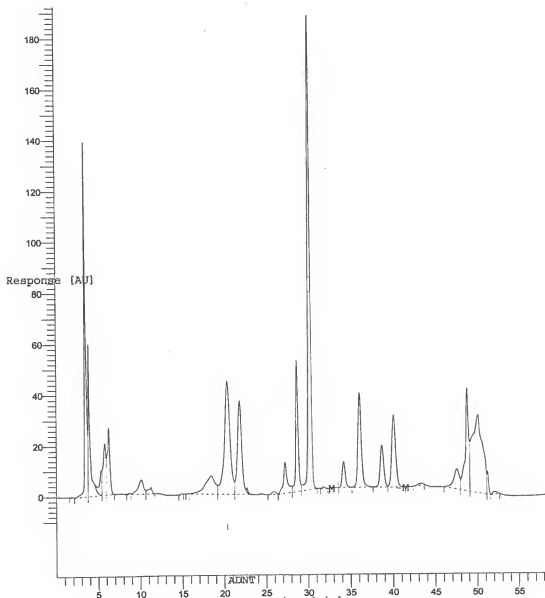


Figure H-2. HPLC Chromatogram showing TNT (36.05 min), 4ADNT (38.67 min), 2ADNT (40.06 min), and an unknown metabolite (34.15 min), 120 minutes after treatment of a non-acclimated sludge solids anaerobic reactor with 100 ppm TNT.

Sample Name :
Filename : D:\GEORGE\2000\DATCD44.RAW
Method :
Start Time : 0.00 min
Scale Factor: 0.0

End Time : 60.00 min
Plot Offset: 289 mAU

Sample #: 44
Date : 4/25/01 09:12 PM
Time of Injection: 6/12/00 11:38 AM
Low Point : 289.50 mAU
Plot Scale: 199.3 mAU
Page 1 of 1
High Point : 488.81 mAU

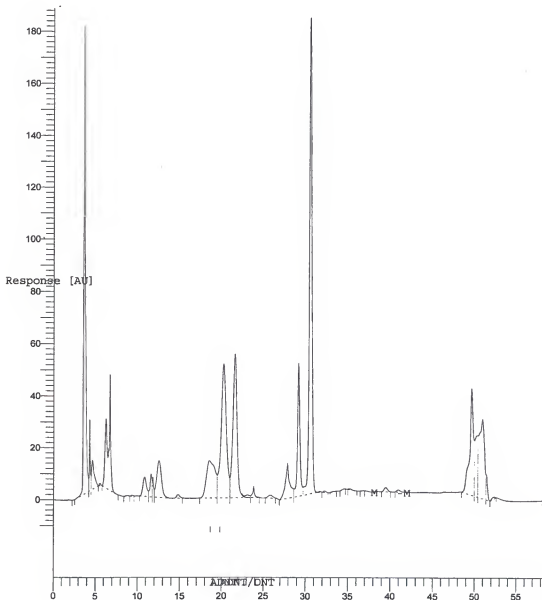


Figure H-3. HPLC Chromatogram showing complete disappearance of TNT and unknown metabolite with minor 4ADNT (38.67 min) and 2ADNT (40.06 min) peaks, 960 minutes after treatment of a non-acclimated sludge solids anaerobic reactor with 100 ppm TNT.

Sample Name :
Filename : 0:\GEORGE\2000\DATA074.RAW
Method :
Start Time : 0.00 min
Scale Factor : 0.0

End Time : 35.00 min
Plot Offset : 290 mAU

Sample #: 74
Date : 4/25/01 09:14 PM
Time of Injection: 6/8/00 07:39 AM
Low Point : 290.07 mAU
Plot Scale: 190.8 mAU
Page 1 of 1
High Point : 460.82 mAU

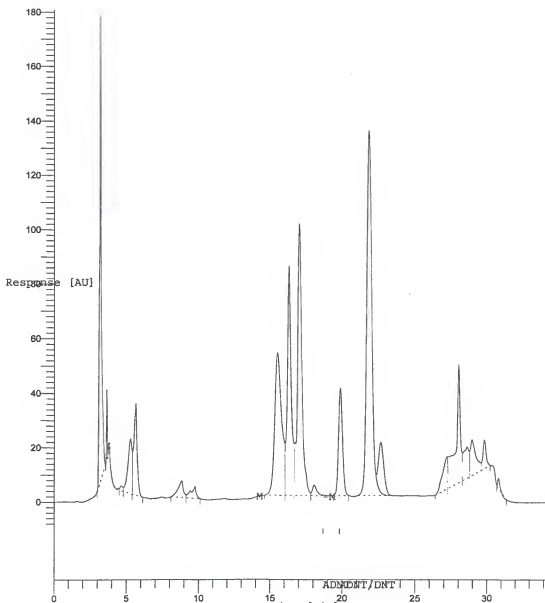


Figure H-4. HPLC Chromatogram showing RDX peak (17.00 min), one minute after treatment of a non-acclimated sludge solids anaerobic reactor with 100 ppm RDX.

Sample Name :
FileName : 0:\GEORGE\2000\DATC057.RAW
Method :
Start Time : 0.00 min
Scale Factor: 0.0

End Time : 29.00 min
Plot Offset: 287 mAU

Sample #: 57
Date : 4/25/01 09:14 PM
Time of Injection: 6/12/00 09:26 PM
Low Point : 286.85 mAU
Plot Scale: 177.1 mAU

Page 1 of 1

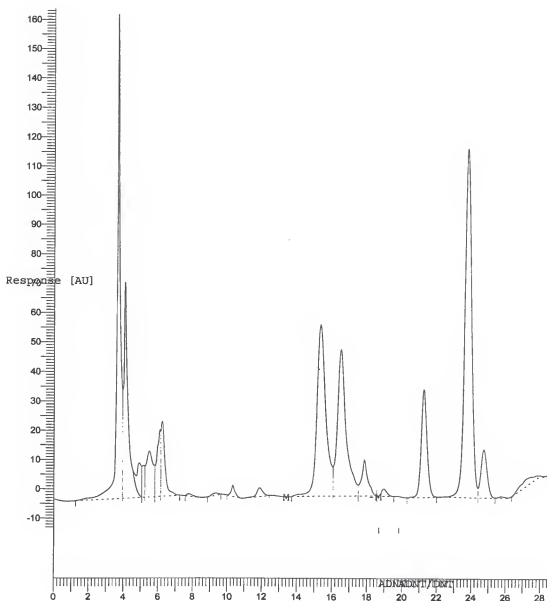


Figure H-5. HPLC Chromatogram showing a minor RDX peak (17.85 min), 960 minutes after treatment of a non-acclimated sludge solids anaerobic reactor with 100 ppm RDX.

Sample Name :
FileName : D:\GEORGE\2000\DATA\78.RAW
Method :
Start Time : 0.00 min End Time : 35.00 min
Scale Factor: 0.0 Plot Offset: 291 mAU

Sample #: 78 Page 1 of 1
Date : 4/25/01 09:15 PM
Time of Injection: 6/8/00 10:05 AM
Low Point : 290.62 mAU High Point : 471.57 mAU
Plot Scale: 181.0 mAU

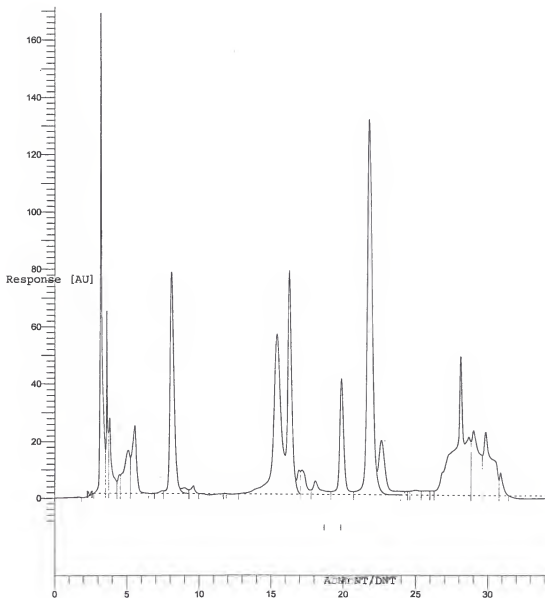


Figure H-6. HPLC Chromatogram showing a minor HMX peak (8.05 min), one minute after treatment of a non-acclimated sludge solids anaerobic reactor with 100 ppm HMX.

Sample Name :
FileName : 0:\GEORGE\2000\DATE037.RAW
Method :
Start Time : 0.00 min
Scale Factor: 0.0

End Time : 30.00 min
Plot Offset: 283 mAU

Sample #: 37
Date : 4/25/01 09:15 PM
Time of Injection: 6/14/00 05:23 PM
Low Point : 283.19 mAU
Plot Scale: 336.0 mAU

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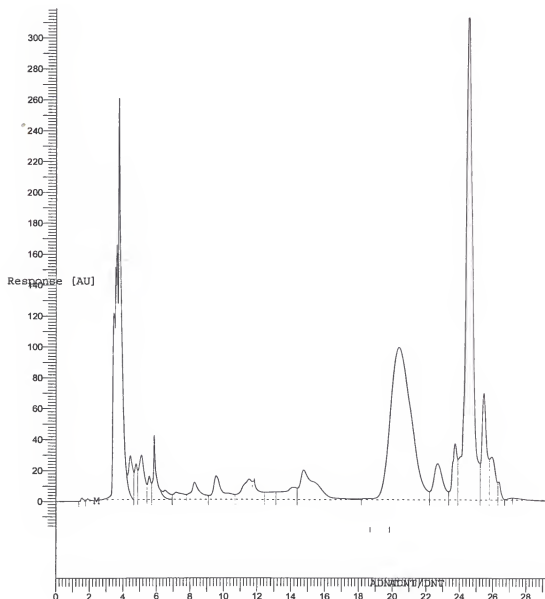


Figure H-7. HPLC Chromatogram showing a minor HMX peak (9.54 min), 2880 minutes after treatment of a non-acclimated sludge solids anaerobic reactor with 100 ppm HMX.

Sample Name :
FileName : D:\GEORGE\2000\DATA092.RAW
Method :
Start Time : 0.00 min
Scale Factor: 0.0

End Time : 70.00 min
Plot Offset: 291 mAU

Sample #: 92
Date : 4/25/01 09:15 PM
Time of Injection: 6/8/00 07:18 PM
Low Point : 290.60 mAU
Plot Scale: 175.8 mAU
High Point : 466.42 mAU

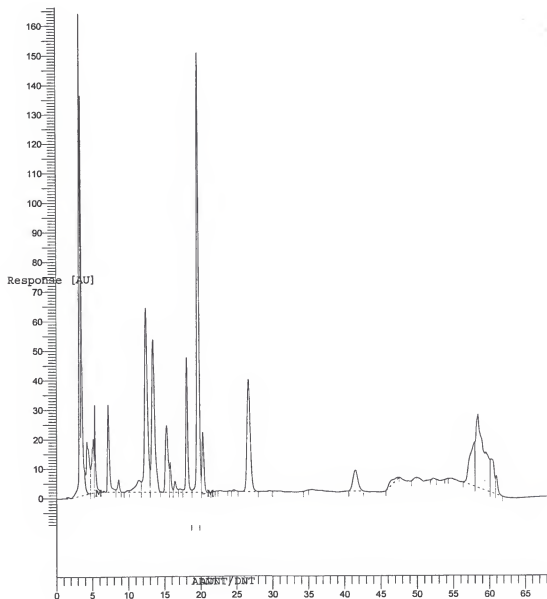


Figure H-8. HPLC Chromatogram showing a TNT (26.62 min), RDX (15.26 min) and HMX (6.56 min) peaks one minute after treatment of a non-acclimated sludge solids anaerobic reactor with 33.3 ppm TNT, 33.3 ppm RDX and 33.3 ppm HMX.

Sample Name :
Filename : D:\GEORGE\2000\DATA098.RAW
Method :
Start Time : 0.00 min
Scale Factor: 0.0

End Time : 60.00 min
Plot Offset: 290 mAU

Sample #: 98
Date : 4/25/01 09:16 PM
Time of Injection: 6/8/00 09:58 AM
Low Point : 289.75 mAU
High Point : 486.65 mAU
Plot Scale: 196.9 mAU

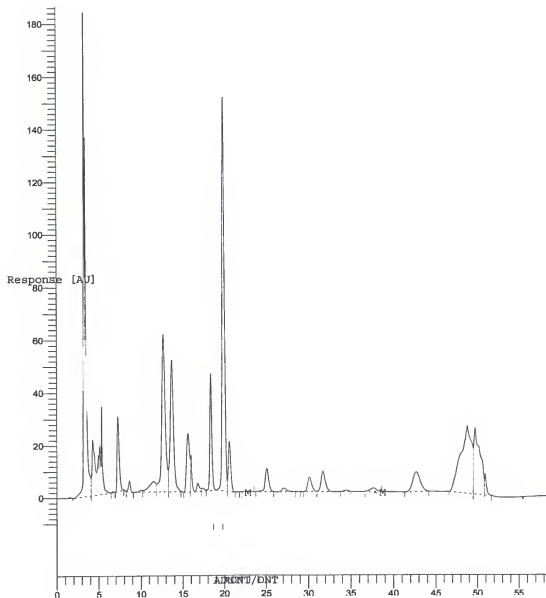


Figure H-9. HPLC Chromatogram showing TNT (27.07 min), unidentified TNT metabolite (25.05 min), 4ADNT (30.11 min), 2ADNT (31.70 min), RDX (15.62 min) and HMX (6.61 min) peaks 40 minutes after treatment of a non-acclimated sludge solids anaerobic reactor with 33.3 ppm TNT, 33.3 ppm RDX and 33.3 ppm HMX.

Sample Name :
Filename : D:\GEORGE\2000\DATE014.RAW
Method :
Start Time : 0.00 min
Scale Factor : 0.0

End Time : 46.00 min
Plot Offset: 169 mAU

Sample #: 14
Date : 4/25/01 09:16 PM
Time of Injection: 6/14/00 08:50 AM
Low Point : 168.88 mAU
Plot Scale: 185.9 mAU
Page 1 of 1
High Point : 354.74 mAU

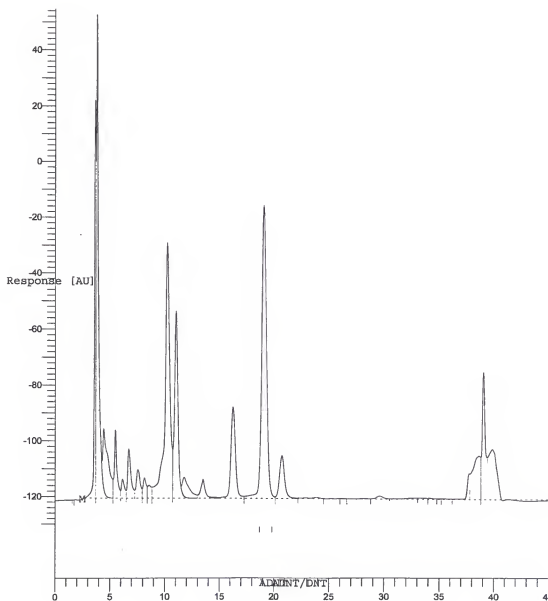


Figure H-10. HPLC Chromatogram showing absence of TNT and metabolite peaks, and RDX (16.22 min) and HMX (6.73 min) peaks 360 minutes after treatment of a non-acclimated sludge solids anaerobic reactor with 33.3 ppm TNT, 33.3 ppm RDX and 33.3 ppm HMX.

APPENDIX I
SAMPLE MINITAB ANALYSES

Table I-1. Two sample minitab output comparing TNT acclimated versus non-acclimated sludge solids for the TNT treatment.

Two sample T for Acclimated vs Unacclimated

	N	Mean	StDev	SE Mean
Acclimat	3	60.50	3.77	2.2
Unacclim	3	62.17	4.54	2.6

95% CI for μ Acclimat - μ Unacclim: (-11.1, 7.8)

T-Test μ Acclimat = μ Unacclim (vs not =): T = -0.49 P = 0.65 DF = 4 Both use Pooled StDev = 4.17

Table I-2. Two sample minitab output for total BMP gas production at 90 days comparing TNT versus control treatments in systems receiving non-acclimated sludge solids.

Two sample T for TNT vs Control

	N	Mean	StDev	SE Mean
TNT	6	61.33	3.84	1.6
Control	6	275.50	3.63	1.5

95% CI for μ TNT - μ Control: (-219.0, -209.4)

T-Test μ TNT = μ Control (vs not =): T = -99.20 P = 0.0000 DF = 10 Both use Pooled StDev = 3.74

Table I-3. Regression analysis for comparison of TNT treatments in high anaerobic solids assays receiving TNT acclimated versus non-acclimated sludge solids.

FULL MODEL

The regression equation is

$$\text{TNT} = 80.2 - 12.2 \text{ LN}(\text{TIME}) - 10.9 \text{ SLUDGE} - 3.86 \text{ LN}(\text{TIME}) * \text{SLUDGE}$$

Predictor	Coef	StDev	T	P
Constant	80.199	2.098	38.22	0.000
LN(TIME)	-12.1881	0.5276	-23.10	0.000
SLUDGE	-10.897	3.102	-3.51	0.002
LN(TIME)	-3.8592	0.9186	-4.20	0.000

S = 4.308 R-Sq = 98.1% R-Sq(adj) = 97.8%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	19102.9	6367.6	343.03	0.000
Residual Error	20	371.3	18.6		
Total	23	19474.2			

Source	DF	Seq SS
LN(TIME)	1	16109.5
SLUDGE	1	2665.8
LN(TIME)	1	327.6

REDUCED MODEL

The regression equation is

$$\text{TNT} = 84.4 - 13.5 \text{ LN}(\text{TIME}) - 21.5 \text{ SLUDGE}$$

Predictor	Coef	StDev	T	P
Constant	84.362	2.477	34.06	0.000
LN(TIME)	-13.4613	0.5783	-23.28	0.000
SLUDGE	-21.524	2.405	-8.95	0.000

S = 5.769 R-Sq = 96.4% R-Sq(adj) = 96.1%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	2	18775.3	9387.6	282.07	0.000
Residual Error	21	698.9	33.3		
Total	23	19474.2			

Source	DF	Seq SS
LN(TIME)	1	16109.5
SLUDGE	1	2665.8

Table I-4. Data set for Minitab regression analysis

Time	TNT	SLUDGE	LN (TIME) *SLUDGE	LN (TIME)
1	74.70	0	0.00	0.00
5	66.20	0	0.00	1.61
10	60.90	0	0.00	2.30
30	38.80	0	0.00	3.40
120	23.00	0	0.00	4.79
160	17.30	0	0.00	5.08
480	1.10	0	0.00	6.17
1	78.00	0	0.00	0.00
30	39.70	0	0.00	3.40
480	2.60	0	0.00	6.17
1	76.80	0	0.00	0.00
30	40.80	0	0.00	3.40
480	4.70	0	0.00	6.17
1	71.13	1	0.00	0.00
5	49.37	1	1.61	1.61
10	35.24	1	2.30	2.30
30	11.69	1	3.40	3.40
60	0.84	1	4.09	4.09
1	67.63	1	0.00	0.00
60	-0.04	1	4.09	4.09
90	6.20	1	4.50	4.50
1	66.05	1	0.00	0.00
30	12.78	1	3.40	3.40
60	0.18	1	4.09	4.09

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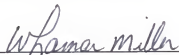
BIOGRAPHICAL SKETCH

George Reinhart was born in Cocoa Beach, Florida, on September 11, 1970. His parent are George and Lois Reinhart and he has younger brother who is studying Dentistry at the University of Florida. His wife is Lynda Marie Reinhart from Navarre Beach, Florida.

George attended high school in Cocoa Beach Florida is a National Merit Scholar. He entered the University of Florida in the fall of 1989 and received a B.S. from the department of Environmental Engineering Sciences in May 1994. He enrolled in graduate school in the Department of Environmental Engineering Sciences at the University of Florida during the summer of 1994.

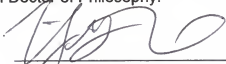
During the winter of 1995 he was surgically treated for malignant embryonal cell testicular cancer. In the summer of 1996 he received chemotherapy in response to metastasis of the disease. He is currently cancer free and living in Alachua County, Florida.

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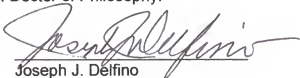
W. Lamar Miller, Chairman
Professor Emeritus of
Environmental Engineering
Sciences

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Timothy G. Townsend
Assistant Professor of
Environmental Engineering
Sciences

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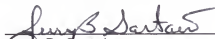
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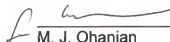
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This dissertation was submitted to the Graduate Faculty of the College of Engineering and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy

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